# INHERITANCE OF RESISTANCE TO HAWAIIAN BEAN RUST (*UROMYCES APPENDICULATUS* (PERS. EX PERS.) UNGER VAR. *APPENDICULATUS*) IN COMMON BEANS (*PHASEOLUS VULGARIS* L.)

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

HORTICULTURE

DECEMBER 1992

By

Manuel A. Balcita

Dissertation Committee:

Richard W. Hartmann, Chairperson
Kent D. Kobayashi
Richard M. Manshardt
Kenneth Y. Takeda
Eduardo E. Trujillo

We certify that we have read this dissertation and that, in our opinion, it is satisfactory in scope and quality as a dissertation for the degree of Doctor of Philosophy in Horticulture.

DISSERTATION COMMITTEE

Richard W. Hartmann
Chairperson

Eduardo E. Trujillo

Kenneth Y. Tekeda

Richard M. Wheeler

Kent D. Kobayashi

## ACKNOWLEDGEMENTS

I express my deep sense of gratitude to my adviser, Dr. Richard W. Hartmann, for his valuable guidance, constant help, and encouragement throughout the course of this study.

Appeciation is also extended to Drs. Kent D. Kobayashi, Richard M. Manshardt, Kenneth Y. Takeda and Eduardo E. Trujillo for their help and useful comments.

I also gratefully acknowledge Dr. J.R. Stavely of the USDA Microbiology and Plant Pathology Laboratory, Beltsville, Maryland for his ungrudging help and hospitality during my visit to his laboratory.

I am also thankful to the staff members of the Poamoho Experimental Station and of the Magoon Greenhouse Facility for their invaluable assistance extended to me during my field and greenhouse plantings and to Budiman Tamimi for his computer expertise.

I deeply appreciate the assistance provided by the Department of Horticulture and by the Environment and Policy Institute of the East West Center to pursue my Ph.D. studies at the University of Hawaii.

Lastly, I extend my gratitude to my parents and sister, Ching, for their patience and moral support.

## ABSTRACT

Bean rust, as caused by *Uromyces appendiculatus* (Pers. ex Pers.) Unger var. *appendiculatus*, is a major fungal disease affecting common beans (*Phaseolus vulgaris* L.) in many tropical and sub-tropical regions of the world. One major characteristic of the fungal pathogen is its high degree of pathogenic variability with over a hundred races identified worldwide. In this study, four different races of bean rust were identified from isolates collected from several locations based on the reactions of a set of nineteen differential bean cultivars.

The inheritance of resistance to the bean rust pathogen was studied using eight of the differentials that showed resistance to one, two, or all of the four rust races, four of the differentials that were susceptible to all four races, two Hawaiian cultivars, and five 'slow rusting' cultivars. Disease inoculation was done with a standardized spore inoculum, and evaluations were made in a greenhouse. Pustules were rated 15 days later on a scale of 1 to 6 based on size. Individual plants were classified on the predominance of pustules of different sizes.

Dominant genes were identified that caused a hypersensitive reaction (but accompanied by pustules also) to two races (called $HR_1$ and $HR_2$) and that caused a resistant reaction (small pustules only) to all four races (called $R_1$, $R_2$, $R_3$, and $R_4$). There were at least two different $R_1$ genes, at least three different $R_2$ genes, at least two different $R_3$ genes, and at least three different $R_4$ genes. The HR genes were epistatic to the R genes. Actopan x Sanilac 37 had HR genes for races H1 and H2. Ecuador 299, NEP-2, and Mexico 235 had an HR gene for race H1 only. These parents and Mexico 309 and Compuesto Negro Chimaltenango had R genes for all the races for which they did not have an HR gene. CSW 643 had R genes for races H2 and H4. Kentucky Wonder 765 and Royal Red had R genes for race H2 only.

All 12 genes identified in all the resistant parents seemed linked into one gene complex, although crossing over (usually $< 10\%$) was observed between genes for resistance to different races as well as between different genes for resistance to the same race.

# TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

## LIST OF TABLES

## LIST OF TABLES (Continued)

## LIST OF TABLES (Continued)

# LIST OF FIGURES

# 1. INTRODUCTION

Bean rust, caused by *Uromyces appendiculatus* (Pers. ex Pers.) Unger var. appendiculatus (*syn. U. phaseoli* (Reben) Wint.), is a major disease of snap and dry beans (*Phaseolus vulgaris* L.). It was first reported in Germany in 1795 (Zaumeyer and Thomas, 1957) and has since then been reported in almost all parts of the world. Besides in the United States, it has also caused severe losses in Canada (Bernier and Conner, 1982), Egypt, Turkey (Rudolph and Baykal, 1978), Kenya (Omunyin et al., 1984), and Latin America (Stavely and Steinke, 1985). Crop losses were as much as 80-100% (Stavely and Steinke, 1985) and losses as much as $250 million nationwide (De Quattro, 1992).

The rust pathogen is a basidiomycete that produces pustules on the upper and lower leaf surfaces and sometimes on the pods (Zaumeyer and Thomas, 1957) (Figure 1). Since the photosynthetic organs are affected, plant yield is consequently reduced (Franje and Quebral, 1980). The pathogen has a high degree of pathogenic variability; several races can occur in a single field collection. Over 150 pathogenic races of this rust fungus have been identified worldwide (Stavely and Steinke, 1985), but it is not possible to tell how many duplicates are included because of the lack of a system for comparing the identifications by different rust workers.

Control measures for this disease include sanitation measures, biological control, chemical control, and host plant resistance. Sanitation measures help, but are often overcome by favorable conditions for the pathogen. Biological control measures have some potential for the future. Chemical control measures are effective, especially if used during early development, but are subject to increasing environmental restrictions. Thus, host plant resistance is increasingly desired as the primary control measure.

Figure 1
Bean Rust Pustules on Bean Leaves and Pods

Resistance is an effective control measure despite high variability in *U. appendiculatus*. Most bean cultivars tested in the IBRN (International Bean Rust Nursery), USBRN (Uniform Snap Bean Rust Nursery), and UDBRN (Uniform Dry Bean Rust Nursery) were resistant to at least one or a few races, while only a few cultivars were resistant to most or all races, but these were generally poor horticulturally. Most currently-grown resistant cultivars possess only a few simply-inherited genes for specific (vertical) resistance against a limited number of pathogen races (Hill et al., 1990). Some new lines, however, like the BARC-Rust Resistant-2 through -18, are resistant to 40 races (Stavely, 1988).

Success with host plant resistance, however, greatly depends on having information on the variability of the rust fungus and the modes of inheritance of resistance in the host. By combining specific resistance genes and various factors contributing to nonspecific resistance, effective and stable genetic control of bean rust may be achieved. Integration with other control measures can also assist in achieving long-lasting protection against bean rust.

Therefore, the objectives of this study were:

1) To isolate and identify several bean rust races of *Uromyces appendiculatus* in Hawaii;

2) To determine the patterns of inheritance of resistance to this disease and to find the relationship between race-specific genes and other factors that are not race-specific.

# 2. REVIEW OF LITERATURE

## 2.1 THE BEAN RUST PATHOGEN

### 2.1.1 Taxonomy

*Uromyces appendiculatus* (Pers. ex Pers.) Unger var. *appendiculatus* (syn. *Uromyces phaseoli* (Reben) Wint.)(bean rust) belongs to the Order Uredinales. All major taxonomic schemes include this rust in the class Basidiomycetes and subclass Heterobasidiomycetidae (Hiratsuka and Sato, 1982). It should not be confused with *Uromyces vignae* Barcl. which was originally named *U. appendiculatus* Pers., a rust first reported in 1921 on *Vigna unguiculata* (L.) Walp. (cowpea). This cowpea rust is now called *Uromyces vignae* Barcl. or *Uromyces phaseoli* (Pers.) Wint. var. *vignae* (Gjærum, 1985).

*Uromyces* is similar to *Puccinia*, both being in Family Pucciniaceae, differing only in their teliospores. In *Uromyces* they are simple, in *Puccinia* compound, formed of two teliospores closely united in a row (Bessey, 1950). Their separation into different genera is maintained for convenience and historical reasons since making them synonymous would entail many nomenclatural changes with no real benefit (Cummins and Hiratsuka, 1983).

Species of the rust fungi (Uredinales) parasitize monocots and dicots throughout the world. *Uromyces* is the second largest genus of rust fungus with several economically important species: *U. appendiculatus* (bean rust), *U. pisi* (DC) Otth. (pea rust), *U. striatus* Schroet. (alfalfa rust), *U. betae* (Pers.) Tul. (beet rust), and *U. dianthi* (Pers.) Niessl (carnation rust) (Cummins and Hiratsuka, 1983).

*Uromyces appendiculatus* infects many *Phaseolus* species such as *Phaseolus acutifolius* A. Gray var. *latifolius* (tepary bean), *P. adenanthus* G. Meyer, *P. anisotrichus* Schlecht., *P. coccineus* L. (runner bean), *P. lunatus* L. (lima bean), *P. obvallatus* Schlecht., *P. polystachus* L., *P. retusus* Walp., *P. sinuatus* Torr. & Gray,

and *P. vulgaris* L. (common bean), as well as *Vigna unguiculata* (L.) Walp. (cowpea), *V. repens* (L.) Kuntze, *V. vexillata* (L.) A. Rich, and *V. radiata* (L.) Wilczek (mungbean) (Vargas, 1980). Although *P. vulgaris* is considered the main host, severe infection has been observed on *P. acutifolius* var. *latifolius* and some varieties of *P. lunatus*, and mild infection on a number of other hosts. *P. vulgaris* L. var. *nanus* (Jusl.) Aschers. (dwarf bean) is less susceptible, and some sub-varieties of *P. multiflorus* Willd. are almost immune (Eriksson, 1930).

### 2.1.2 Life Cycle

*U. appendiculatus* is an automacrocyclic obligate parasite, that is, it has its life cycle confined to a single host, it has all spore states, and it will not grow in vitro (Hiratsuka and Sato, 1982; Vargas, 1980).

The urediniospores (summer spores) are repeating vegetative spores produced by dikaryotic mycelia. These spores can germinate immediately upon maturity and initiate new dikaryotic mycelia. Under favorable conditions, they produce another generation of the same kind of spores from uredinia in about 10-15 days (Zaumeyer and Thomas, 1957). This rapid increase on a host several times during the growing season makes this the most destructive spore state (Hiratsuka and Sato, 1982). These urediniospores are usually spread by wind or water over great distances causing great epidemics.

Later in the season, teliospores (winter spores) are produced from telia and serve to carry the organism through the winter months. Teliospores are first dikaryotic, but karyogamy occurs to produce diploid nuclei in the spores (Hiratsuka and Sato, 1982). Light intensity seems to influence production of the teliospores because they are not produced at all in Hawaii. In the northern regions, they are not usually produced in late spring and early summer when the days are the longest and the light more intense, but only later in the season (Zaumeyer and Thomas, 1957). They have a dormancy period and do not germinate until the next growing season to produce basidia that form

haploid basidiospores. In studies made by Gold and Mendgen (1983a), light intensities of 15,000-26,000 lux supported teliospore germination and basidiospore formation with an optimum of 17,000 lux, but there was a need for light-dark alternation or the phase change from light to dark.

The basidiospores infect bean leaves and produce spermagonia in about six days at 22-26°C. Haploid gametes or spermatia are produced from the spermagonia after approximately seven days (Zaumeyer and Thomas, 1957).

Cross fertilization of the opposite mating types results in aecium formation, but both phenomena are rarely observed in nature. The resulting aeciospores are nonrepeating vegetative spores produced as the result of dikaryotization (Hiratsuka and Sato, 1982). They are able to infect bean plants upon their release and 8-10 days later produce a pustule with urediniospores. These spores germinate to provide hyphae that infect the plant and form new pustules wherein new urediniospores and eventually teliospores may develop (Vargas, 1980).

Leaf infection by urediospores of *U. appendiculatus* follows a typical sequence for rust, that is, spore germination; formation of infection structures; formation of infection hyphae; formation of a haustorial mother cell; and finally, formation of haustoria.

The onset of germination of the urediospore depends on the absence of self-inhibitors and the presence of endogenous stimulators that overcome any inhibition and allow growth of the germ tube. A hexosamine, probably glucosamine, has been reported to be the reactive component of the urediospore (Kaminskyj and Heath, 1982). Cyclic adenosine monophosphate (cAMP) or its derivatives induce nuclear division in the spore as well as in subsequent infection structures (Hoch and Staples, 1984). Other endogenous stimulators are saturated and unsaturated methyl ketones with 6-9 carbons or cyclic unsaturated ketones (Wolf, 1982).

The presence of a self-inhibitor chemical, identified as methyl-cis-3, 4 dimethoxycinnamate (MDC) was also reported by Wolf (1982). MDC prevents pore plug dissolution by specifically blocking the hydrolytic enzymes involved in the degradation of cell wall material. When spores are present in dense populations, either in pustules or in suspension, this inhibitor prevents germination or reduces the germination rate. This self-inhibition is undoubtedly advantageous to the rust since it prevents premature germination in the pustule and thus contributes to efficient spore dispersal.

Germ tube growth is a contact response of the fungus to the stomate or leaf surface. Wynn (1976) showed that up to 92% of the rust spores formed appressoria above the stomates that serve as the fungal penetration sites. Such a thigmotropic response may involve extracellular proteins that bind the germ tube to an inductive surface, and this binding may be necessary for the induction of infection structures (Epstein et al., 1985).

There are also indications of involvement of gas exchange in stomate recognition. Alten (1983) stated that the oxygen and carbon dioxide coming out of the stomates can serve as signals for recognition. The $CO_2$, via the carbonic cycle, alters the pH of the fluid to which the germ tubes react, and the germ tubes may orientate in a simple pH-gradient.

Stomatal recognition by stimulus receptors in the germ tube is followed by nuclear division induced by the depolymerization of the cytoplasmic microtubules and microfilaments in the germ tube (Staples and Hoch, 1982). Growth of the germ tube is generally at right angles to the ridges of epidermal cells that encircle the stomates (Wynn, 1976).

The germ tube subsequently forms an appressorium over a stomate through which an infection peg penetrates the leaf (Pring, 1980). Mendgen (1973) described the

appressorium as having a dense cytoplasm, with walls containing dark, granular deposits, that seems to mold itself into the fissures and folds of the outer ledge of the stomate. With the formation of the appressorium, adenosine is incorporated into nuclear DNA, thus nuclear DNA is not synthesized until the spores differentiate (Staples, 1974). Incorporation of uridine into template RNA also occurs during germ tube differentiation which suggests that formation of infection structures may depend on the synthesis of messenger RNA. After appressorium formation, the amount of template RNA activity declines (Ramakrishnan and Staples, 1970). Ethylene, an endogenous regulator, was reported by Montalbini and Elstner (1977) to play a role in the establishment of the rust fungi based on an observed burst of ethylene production 13 hours after inoculation coinciding with the penetration of the stomates by the germ tube.

An infection peg develops from the appressorium which pushes the guard cells of a stomate apart. After the passage of the fungal cytoplasm through the infection peg, the stomate closes again forming a substomatal vesicle. Meanwhile, the appressorium collapses. Glyoxysomes, lipid bodies, and glycogen particles (ß-particles) were shown by Mendgen (1973) to be numerous in the substomatal vesicle.

Penetration of the cells starts with the formation of a haustorial mother cell and its adhesion to a plant cell. This is followed by the formation of a fungal haustorium within the plant cell (Pring, 1980). The fungus develops infection hyphae and haustoria as it penetrates the host tissue, eventually forming a young pustule. Mendgen (1973) reported that lipids and glyoxysomes are scarce in the haustorial mother cell, the first haustorium, and the secondary hyphae. Energy for fungal differentiation during this infection stage was suggested by Kaminskyj and Day (1984) to come from simple sugars, sucrose, and other complex components in the host tissue.

Infection is favored by incubation in low light intensity for 18 hours (Augustin et al., 1972). Despite this, light is still needed during the inoculation period for successful entry of the fungus since the opening and closing of the stomates, which are the entrance sites of the germ tubes, are affected by light. After incubation, as long as the infected plants receive at least a 12-hour photoperiod, sporulation is expected to increase (Cohen and Rotem, 1970).

### 2.1.3 **Morphology**

The most commonly observed spore forms are the urediniospores and teliospores. The light brown urediniospores have a short hyaline pedicel and are produced in rows within pustules on the upper and lower surfaces of the leaf. They are one-celled (Figure 2); globoid to ellipsoidal in outline but shaped similar to a doughnut, and measure about 8-24 microns (ave. 22.5 microns) by 20-37 microns (ave. 28 microns) (Zaumeyer and Thomas, 1957). The walls are thin and spiny with the spines situated in small, circular depressions on the spore surface, each surrounded by a slightly raised annulus. Two equatorial or superequatorial germ pores are found on opposite sides of the spore, and these sunken areas constitute regions of thicker spore walls towards the interior and reduced surface ornamentation (Hartwick et al., 1975).

The dark brown teliospores develop from successive new growing points on sporogenous cells, being equivalent to sympodioconidia (Muller et al., 1974). They are one-celled; globoid to broadly ellipsoidal in shape; range in width from 20-28 microns (ave. 24 microns) and in length from 25-35 microns (ave. 30 microns), and have a short hyaline pedicel (Zaumeyer and Thomas, 1957). The wall is uniformly thick with no ornamentation on the spore surface (Muller et al., 1974).

A teliospore may germinate to produce a basidium which has crosswalls that divide it into four cells, each of which produces a basidiospore. These basidiospores are small, delicate, and short-lived and produce haploid mycelia that form small,

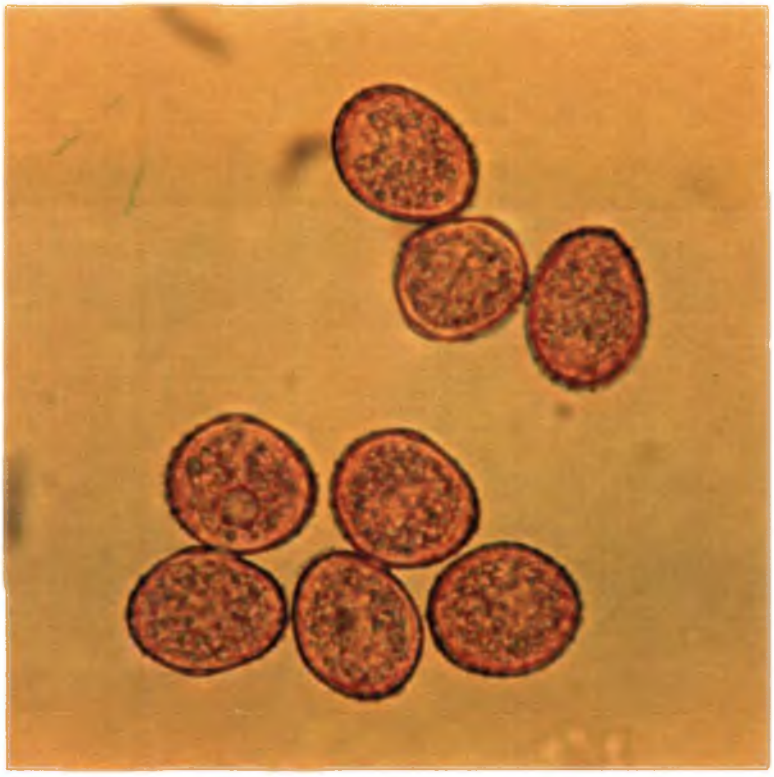

Figure 2
Bean Rust Urediniospores

chlorotic, epiphyllous flecks called spermagonia (Zaumeyer and Thomas, 1957). After a few days, the spermagonia contain small, single-celled spermatia, receptive hyphae, and insect-attracting nectar (Savile, 1979).

Aecia, caused by the cross fertilization of a sperm cell with a receptive hypha of the opposite mating type, primarily occur on the lower leaf surface although they may also form occasionally on the upper surface. The white aecia are cupulate and occur in groups (Zaumeyer and Thomas, 1957). They are also described as aecidioid aecia due to the presence of surrounding structures called peridia which are made of specialized spores (Hiratsuka and Sato, 1982). The resulting aeciospores are ellipsoidal, 16-20 microns by 20-26 microns, thick-walled (1-1.5 microns), colorless, and minutely verrucose (Arthur, 1962).

### 2.1.4 Symptomatology

Bean rust infects most aerial organs of the plant although it is most abundant and conspicuous on the leaves. Initial infection can occur on either leaf surface but the symptoms are usually seen first about 5-6 days after inoculation on the lower leaf surface as minute, whitish, slightly raised spots (sori). The germ tubes enter the stomates and grow within the stomatal and intercellular spaces resulting in the enlargement of the sori to form mature reddish-brown pustules which rupture the epidermis and may attain a diameter of 1-2 mm within 10-12 days after inoculation. The rust-colored urediospores first appear in these pustules (Figure 3). Secondary and tertiary pustules may also develop around the primary pustules and may merge eventually with the original pustule. The entire infection cycle occurs within 10-15 days after which the urediospores are passively released and dispersed. As the plant becomes older and the leaves somewhat moribund, teliospores replace the urediospores except under long days and in tropical regions where they are rarely present. As the

Figure 3
Symptom Development of Bean Rust

*Left*, uninoculated leaf; *Middle*, sori observed at 5-6 days; *Right*, mature pustules after 14 days

dark-brown teliospores replace the urediospores, the sori gradually become black (Zaumeyer and Thomas, 1957).

Rust rarely kills a bean plant, but it robs the plant of water and nutrients resulting in a debilitated plant with fewer pods (De Quattro, 1992).

### 2.1.5 **Pathogenic Variability**

The bean rust pathogen exhibits high pathogenic variability with several races sometimes occurring in a single field collection. Over 150 pathogenic races of *U. appendiculatus* have been identified wordwide. Table 1 shows a list of some countries with the number of rust races collected. However, there is difficulty in comparing data on pathogenic variability from different locations because different rating scales and sets of differential cultivars are often used.

Table 1
Number of Races Reported in Different Countries

| Country | No. of Races | References |
|---|---|---|
| Africa (East) | 8 | Vargas, 1980 |
| Australia | 18 | Ballantyne, 1976 |
| Brazil | 39 | Vargas, 1980 |
| Colombia | 10 | Vargas, 1980 |
| Costa Rica | 11 | Vargas, 1980 |
| El Salvador | 5 | Vargas, 1980 |
| Guatemala | 7 | Vargas, 1980 |
| Honduras | 5 | Vargas, 1980 |
| Jamaica | 21 | Shaik, 1985a |
| Mexico | 31 | Vargas, 1980 |
| Netherlands | 2 | Hubbeling. 1957 |
| New Zealand | 4 | Yen and Brien, 1960 |
| Nicaragua | 4 | Vargas, 1980 |
| Peru | 4 | Vargas, 1980 |
| Puerto Rico | 12 | Vargas, 1980 |
| Tanzania | 9 | Mmbaga and Stavely, 1988 |
| U.S.A | 58 | Stavely et al., 1992 |
| Western Oregon | 1 | Vaughan, 1974 |
| Hawaii | 2 | Parris and Matsuura, 1941 |

Much time and money is spent each year conducting bean rust race surveys. The races are identified by arbitrarily assigned numbers or letters, usually in order of identification, with each race number or letter corresponding to a unique pattern of responses among a specific set of differentials. Unfortunately, such race designations cannot be used outside their areas of immediate application. Some taxonomic schemes have been proposed such as race names based on the resistant varieties or the resistance genes they carry (Day, 1973). Adoption of a common scheme would provide physiologic race designations that are international and would enable farmers, plant breeders, or plant pathologists to know which races of a pathogen are a threat no matter where they occur.

## 2.2 FACTORS AFFECTING THE BEAN RUST DISEASE

### 2.2.1 **Temperature**

The presence or absence of bean rust under natural conditions is strongly influenced by temperature. Although spores will germinate at 10°C, optimum germination occurs at 18°C (Gold and Mendgen, 1983b) to 20°C (Zaumeyer and Thomas, 1957). The optimal temperature for disease development is 15-20°C for the pre-penetration stage and 24-26°C for the post-penetration stage (Code et al., 1985). As temperatures increase above the 20°C optimum, germ tubes exhibit disorientation and failure to penetrate the host (Alten, 1983), making susceptible host plants appear partially resistant (Schein, 1961).

Spores have been stored in laboratories successfully at very low temperatures. Although infectivity was maintained, germination was reduced and mutations for pathogenicity have been observed. Schein (1962) reported a viability of 40% for spores stored at -60°C for 670 days without impairment of their infectivity. Zaumeyer

and Thomas (1957) reported induced pathogenic mutations in spores stored at -18°C for 5-7 months or at 0°C for one year.

Unusually high temperatures have also been used, primarily to kill rust mycelia in infected host tissue (Zaumeyer and Thomas, 1957; Farina et al., 1981). However, heat treatments like 30-32°C for 3-4 days have also proven to be effective in activating teliospore germination although spore mortality then increased (Gold and Mendgen, 1983b).

Yarwood (1978) suggested using a translocated heat therapy to cure host tissue of bean rust infection. The distal halves of rust-inoculated bean leaves were heated to 75°C for 10 sec. Although the heated tissue was killed, most of the fungi in the proximal halves of the same leaves were killed without permanent damage to the proximal leaf tissue. Dipping the leaves in 45°C or in ice water for 10 sec. immediately after heat treatment further reduced the amount of the fungus in the nonheated tissue.

### 2.2.2 Moisture

Infection by *U. appendiculatus* is favored by prolonged periods (10-18 hours) of moisture conditions greater than 95% RH, but rarely occurs at humidities below 95%. It is for this reason that rust rarely occurs in the dry parts of California, but occurs annually, even as an epidemic, in the humid parts (Zaumeyer and Thomas, 1957). In Hawaii, over the years, many reports of bean rust occurrence have come from rainy areas like Hilo, Kona and Kula (Plant Disease Clinic Report, 1968-1982). It was reported that the greatest number of spores are released during dry (less than 60% RH) days which were preceded by a long dew period or rain the previous night (Vargas, 1980).

Water-infiltrated bean leaves have been reported to be more rust resistant than normal leaves. The infiltrated condition did not interfere with stomatal penetration,

and formation of appressoria and substomatal vesicles, but subsequent infection hyphae were rare (Zaumeyer and Thomas, 1957).

### 2.2.3 Host Factors

The age of leaves, leaf type, physiological condition of the host, and cultivar type influence rust spore production. Alten (1983) asserted that leaf age did not affect the germination of urediospores, but studies made by Imhoff et al. (1981) found that spores from old leaves and old pustules germinated only 2/3 as well as those from young materials.

Leaf age definitely affects infection frequency. The fastest infection occurs in leaves that are a few days old due to a greater number of stomates per unit area. In adult leaves, only a few germ tubes manage to penetrate a stomate to get into the mesophyll. However, once the germ tube reaches the leaf interior, it has the same chance of pustule formation on young or adult leaves (Alten, 1983). Schein (1965) reported low susceptibility of bean leaves to infection at the time of unfolding with increasing susceptibility until the leaves were 20-40% expanded. Thereafter, susceptibility to the pathogen decreased until a very low level was reached when the leaves were fully expanded.

### 2.2.4 Pathogenic Interactions

Various interactions have been observed between infections by *U. appendiculatus* and other bean pathogens and non-pathogens. According to Vargas (1980), rust infection may predispose plants to subsequent infection by bean pathogens such as *Pseudomonas phaseolicoli* Dows., *Colletotrichum lindemuthianum* (Sacc, & Magn), and *Thielavopsis basicola* (Berk. and Br.) Ferr. Yarwood (1965) showed that rust-infected leaves were also readily infected with *Sphaerotheca fuliginea* (Schlecht.) Salmon. (powdery mildew). Coinoculation with rust and *Xanthomonas campestris* (Smith) Dye. pv. *phaseoli* (common bacterial blight) on the same foliage resulted in

some interaction whereby rust accentuated the damage caused by the blight pathogen (Zaiter et al., 1990).

Inoculation with tobacco mosaic virus (TMV) and possibly other viruses in rust-infected plants resulted in necrotic rings occurring on the perimeter of the rust pustules. Rust spores are conjectured to contain compounds that inhibit virus multiplication (Vargas, 1980). On the other hand, bean common mosaic virus (BCMV) was shown to reduce rust pustule size (Zaiter et al., 1990).

An interaction of *U. appendiculatus* and *Meloidogyne incognita* (Kofoid & White) (root knot nematode) was observed by Bookbinder and Bloom (1980). While both pathogens suppress shoot and root growth, dual infection resulted in a synergistic effect on the host although at the expense of each other's reproduction. Rust uredia were reduced in size and sporulation capacity while the nematode produced fewer root galls and fewer eggs.

## 2.3 CONTROL OF THE BEAN RUST DISEASE

### 2.3.1 **Cultural Control**

Cultural control recommendations include crop rotation and field sanitation which requires the removal and/or burning of old plant debris that may contain viable spores of the bean rust fungus. Beans should not be planted on land that produced a heavily rust-infected crop the preceding year or close to stacks of old bean straw infested with rust, as the new crop may become infected earlier in the season and more severely than it would have under other conditions (Zaumeyer and Thomas, 1957).

Since bean rust is known to decrease yield more severely if infection occurs before flowering, planting dates may be adjusted for certain production areas to avoid or reduce the incidence of rust infection during the preflowering stage of bean development. Reduced plant density also may decrease rust incidence (Vargas, 1980).

Crowding is a condition that is known to favor growth of the fungus (Martin and Leonard, 1970).

The incidence of the disease may be influenced by different cropping systems used in bean production. For example, rust incidence was lower when beans were grown alone than in association with corn. This lower incidence of the disease may reflect the reduced temperature due to shading by the corn or the higher relative humidity present within the corn-bean canopy (Vargas, 1980) although the possibility of induced rust resistance on beans by maize rust (Allen, 1975) should also be considered.

### 2.3.2 **Biological Control**

2.3.2.1. Hyperparasitism. Numerous fungi have been reported to inhibit the development of rust spores. Allen (1982) reported the potential use of *Verticillium lecanii* (Zimm.) Viegas for biological control of *U. appendiculatus* because it colonizes uredia on inoculated bean seedlings. The hyphae penetrated and invaded the urediospores, but did not lyse them. *Darluca filum* (Biv. Bern. ex Fr.) Cast, the imperfect stage of *Eudarlaca caricis* (Fr.) O. Erikkss., is also a known hyperparasite of the urediospores of many rust species in the tropics. As a parasite of the rust pathogen, it directly penetrates walls of urediospores by mechanical and enzymic processes without forming any specialized penetration structures and causes the disorganization of the cytoplasmic cell content (Buchenauer, 1982). However, the presence of urediospores of *U. appendiculatus* enhances germination and longevity of *D. filum* which can be attributed to chemical compounds from the rust spores (Swendsrud and Calpouzos, 1970).

Baker et al. (1983) also reported an inhibitory effect of *Bacillus subtilis* Coch emend. Prazmowski (APPL-1 isolate) which reduced the number of rust pustules more than 95% when applied in liquid culture prior to inoculation. There was also a reduction in urediospore germination and formation of abnormal germ tubes and

cytoplasmic protrusions. Field tests by Baker et al. (1985) showed a reduction in rust severity with 3 applications/week of *Bacillus subtilis*.

2.3.2.2. Induced Resistance. Resistance can be induced by infection with a non-virulent race (Johnson and Allen, 1975) or with an alien rust fungus such as *Puccinia sorghi* Schw. from maize, *Puccinia striiformis* West. from wheat (Allen, 1975), or *Puccinia helianthii* Schw. from sunflower (Yarwood, 1956). Yarwood (1956) suggested that enzymes released during the germination of the avirulent urediospores induce the synthesis of antifungal compounds responsible for resistance against the virulent rust race (Yarwood, 1956). Such induced resistance may limit disease development where different crops are grown in mixtures as in the intercropping of cereals with legumes as practiced in the tropics (Allen, 1975).

Glucanes isolated from the cell walls of urediospore germ tubes of *U. appendiculatus* were found to be effective as elicitors that induced phytoalexin accumulation and protection against the bean rust fungus as long as they were applied before infection (Hoppe et al., 1980). Glucanes do not affect spore germination and appressoria formation, but cause the destruction of substomatal vesicles and prevent haustorial formation. Although there may still be scattered intercellular growth of the rust fungus, there is no haustorial formation. This is related to the deposition of electron-opaque material between the plasmalemma and cell walls of plant cells at the sites of contact between host and fungal cells (Ebrahim-Nesbat et al., 1982).

### 2.3.3 Chemical Control

Several chemical control methods have been suggested for control of bean rust. Fungicide applications should be made early because bean rust reduces yields more severely when infection occurs before flowering than when it occurs after flowering (Vargas, 1980).

Sulfur dusts or sprays have given good control although their effectiveness is largely dependent on optimum particle size and correct formulation (Buchenauer, 1982). Finely ground (325-mesh or finer) sulfur dust applied at 20-25 lbs/acre is very effective if dusted on beans before any rust is noticeable. Dusting done after the infection has become widespread must be repeatedly done with increased dosages and may not be so effective. Lime sulfur sprays are also effective especially with the addition of zinc sulfate. Lime sulfur solutions may also be used to disinfect old bean poles because of the strong possibility that large numbers of spores adhere to the poles (Zaumeyer and Thomas, 1957). Sulfur causes lysis of germ tubes and at higher concentrations, germination of urediospores is completely inhibited (Buchenauer, 1982).

In field trials under natural infection by *U. appendiculatus*, oxycarboxin (2 liters/hectare) gave the best result, followed by triforine (1.5 liters/hectare) (Rolim et al., 1981). Pring and Richmond (1976) explain that oxycarboxin causes ultrastructural changes in the fungus by causing disruption of the mitochondria and cristae of the haustoria and intercellular hyphae. With postinfectional treatment, oxycarboxin induced cytological changes; the mitochondria in the haustoria swelled, their cristae became disorganized, and the plasmalemma surrounding the haustoria became fragmented. Two days after treatment, the mitochondria became disrupted and after six days, the haustoria and intercellular hyphae were dead (Buchenauer, 1982). Oxycarboxin was found to be superior to carboxin in long-term effectiveness because of its pronounced stability within the plant tissue and on the plant surface (Snel and Edgington, 1969). Carboxin was readily oxidized to the nonfungitoxic sulfoxide (90-92%) and the fungitoxic sulfone (8-10%) in bean plants (Snel and Edgington, 1970).

Other preventive chemicals have been recommended such as Daconil or Chlorothalonil (225g/100 l), Dithane M-22 or Maneb (4-5kg/ha), Manzate D 80W or

Maneb (4kg/ha in 1000 l water), and Dithane M-45 or Mancoseb (3-4kg/ha) (Vargas, 1980). Myclobutanil is also being evaluated for rust control and has the potential for labeling in the near future (Mullins and Bost, 1991). Preinoculation treatments of leaves with triphenylbismuth dichloride at 16 micrograms/ml (Evrard and Lepeivre, 1983) and with triphenylphosphite (TPP) at 125 micrograms/ml (Rusuku et al., 1984) prevented pustule formation. The antibiotic phleomycin was also reported by Small et al. (1961) to be an effective therapeutant and protectant for rust.

Chemical seed treatment is also used to provide protection to the cotyledons and first leaves of the plant from attack by airborne fungi. The uptake of systemic fungicides such as benomyl, thiophanate-methyl, thiabendazole, and fenopronil by bean seeds in the dry state is enhanced by soaking the seeds in solvents like acetone, benzene, or ethanol (Muchovej and Dhingra, 1980).

In the use of these fungicides, Hill et al. (1990) said that sub-lethal application of certain fungicides may result in a yield similar to that obtained with non-specific resistance, and this may involve one or more of the following mechanisms: reduced infection efficiency, reduced lesion size, reduced sporulation, increased length of repeating cycle, etc. Combining different disease reduction mechanisms, whether caused by sublethal fungicide application and/or horizontal resistance, may result in synergistic rather than just additive disease reduction while utilizing less pesticide.

### 2.3.4 **Control by Genetic Resistance**

Many commercial cultivars possess resistance to one or more races, but no cultivar or germplasm source has been found that is immune or resistant to all reported races or populations of rust. A few varieties, however, show a high degree of resistance or at least tolerance to many races. Results from the different rust nurseries (IBRN, USBRN, and UDBRN) through the years, showed that nearly every entry was

susceptible at one or more locations and that cultivars that were formerly resistant in a specific area were later attacked, indicating a continuing shift in pathogenic strains.

Among the cultivars and lines tested, most types showing immunity or high resistance are of Latin American origin (Meiners et al., 1975). Some especially resistant cultivars are 'Compuesto Negro Chimaltenango' (Grafton et al., 1985); G700, NEP-2, and V3249-13-1C (Stavely, 1984a); Costa Rica 1031 and Negro Jalpatagua (Meiners, 1979); Cocacho, Cuilapa 72, Redlands Pioneer, Redlands Greenleaf B and C and Pueblo 87 (International Bean Rust Nursery Results, 1977-1978); Mexico 309 and B-190 (Stavely, 1984b); and P.I. Nos. 151388, 151388, 151395, 151396, 151406, 181996 and 189013 (Stavely, 1988). Cultivars that were susceptible in most locations include BBL 274, Green Isle, Spartan Pride, Strike, and a host of other U.S. cultivars and breeding lines (Meiners, 1980). In screenings of over 3,400 plant introductions (PI), 32 were resistant to all of the 55 U.S. races that were available at that time (Stavely et al., 1992).

Immunity or resistance in beans to a rust race is not rare, but there are many kinds of resistance. However, the resistance of many cultivars is often overcome within a short time due to the frequent changes in the pathogenic diversity of the fungus (Schwartz and Temple, 1978).

2.3.4.1 Specific Resistance. This type of resistance is usually simply inherited and dominant (Vargas, 1980). Specific resistance is more accurately referred to as gene-specific because its expression is dependent on the presence of the corresponding gene(s) for avirulence (McIntosh and Watson, 1982). This gene-for-gene resistance is typically expressed after the first haustorium is formed (Heath, 1981) in the form of pustule-limiting necrotic reactions. These necrotic reactions may either completely prohibit spore production by the fungus (highly resistant) or only reduce pustule size (resistant or moderately resistant) (Stavely, 1984c). The host cell death or

necrosis of the haustorium-containing cells is believed to induce the accumulation of phytoalexins, particularly phaseollin, which is inhibitory to fungal growth (Heath, 1981; El Naghy and Heitefuss, 1976). Sempio et al. (1975) showed that the resistance of the bean variety 814 is due to phenolic compounds that immediately form in cells penetrated by the fungal haustoria and is accompanied by rapid changes in the free amino acids, that is, asparagine decreases while glutamine, lysine, and $\tau$-aminobutyric acid increase. In the susceptible Pinto III, however, asparagine increases while glutamine, lysine, and $\tau$-aminobutyric acid decrease. Actually, there is still controversy whether the necrosis of the haustorium-containing cells is the cause or the consequence of the death of the enclosed haustorium (Heath, 1976).

Another possible explanation for resistance is the impairment of the functioning of the haustorium through the deposition of fibrillar material in the extrahaustorial matrix, development of callose-containing collars around the haustorial necks, and subsequent encasement of the haustoria by continued synthesis of collar material (Heath, 1981, 1982).

Many different types of genetic control of resistance have been reported. Most commonly reported is the presence of single dominant genes that control resistance to one race. Examples of cultivars with single dominant genes for resistance are: BAT 41 (Bean Program Annual Report, 1981), Great Northern 1140 (Augustin et al., 1972), and Cacahuate 72 and PR-5 (Bravo and Galvez, 1976).

Other authors have reported cultivars that carry more than one dominant gene for resistance. The cultivar 1458 was reported to have a single dominant gene to each of five Jamaican races (Carvalho et al., 1978). Another study in Brazil found that resistance to each of five local races depended upon a single dominant gene (Meiners, 1981).

Some studies have reported two dominant genes that are independently inherited. The reported resistance of T-39, Aurora, and Olathe to races 44 and 52 was determined by Grafton et al. (1985) to be controlled by a single dominant gene to each race with all genes involved assorting independently without epistasis. Crosses by Christ and Groth (1982a) showed dominant resistance alleles at two independent loci; one locus governing resistance to isolate SI-5, designated as $Up_1$; and another locus governing resistance to P10-1, designated as $Up_2$. U.S.#3, which is resistant only to SI-5, is homozygous resistant at the $Up_1$ locus, but homozygous susceptible at the $Up_2$ locus. Early Gallatin, which is resistant only to P10-1, is homozygous resistant at the $Up_2$ locus, but homozygous susceptible at the $Up_1$ locus. Pinto III, which is susceptible to both isolates, is homozygous susceptible at both the $Up_1$ and $Up_2$ loci. Kolmer and Groth (1984) showed that the bean line 814 has a single dominant gene producing a minute uredinium type response line to rust isolate SI-5. The $F_2$ of the cross between 814 and the fully susceptible Pinto III segregated in a 3:1 ratio for minute:large uredinia infection types. The $F_2$ of the cross between 814 and Early Gallatin, which is susceptible to SI-5 and hypersensitive to P10-1, segregated in a 9:3:3:1 ratio for resistance to isolates SI-5 and P10-1: resistance only to P10-1: resistance only to SI-5: susceptibility to both isolates.

Various kinds of epistasis have also been reported. Grafton et al. (1985) reported that the resistance of T-39, Aurora, and Olathe to race 44 was being controlled by complementary dominant genes that assort independently. Dominant epistasis explains the resistance in Pompadour Checa (Finke et al., 1985). The $F_2$ segregation of the resistant Pompadour Checa, and the susceptible Great Northern Tara to three cultures of rust showed a good fit to 13:3 resistant:susceptible plants, respectively. They have hypothesized that the resistance is determined by two major genes with a dominant resistance gene exhibiting epistasis. Rust susceptibility is expressed only in the

presence of the dominant allele for susceptibility and homozygous recessive alleles at the other locus.

In the cross between 814, with the dominant gene producing a minute uredinium type of response, and U.S.#3, which has a dominant gene producing a large, sometimes sporulating fleck with SI-5, the $F_2$ segregated in a ratio of 12 with minute, non-necrotic uredinia grading into minute necrotic flecks: 3 with sporulating uredinia surrounded by large areas of necrosis: and 1 with large non-necrotic uredinia. The gene conditioning the minute uredinium infection type from 814 appears to be epistatic to the gene conditioning the necrotic fleck from U.S.#3 (Kolmer and Groth, 1984).

Single recessive genes have also been reported to be responsible for the small pustule of G 05066 and the necrotic pustule of BAT 153 (Bean Program Annual Report, 1981).

Linkage groups of numerous dominant genes that confer resistance to large numbers of races have also been reported. Resistance of B-190, whose resistance was derived from Mexico 309, appears to be conditioned by 17 dominant genes, one per race, that are linked in coupling (Stavely and Steinke, 1985). L226-10 and L227-1 were also reported to have large numbers of genes in a linkage group providing resistance to most indigenous races of rust prevalent in the U.S., Puerto Rico, and Dominican Republic (Freytag et al., 1985). BARC-Rust Resistant-2 through -18 have at least one resistance gene for each of the 42 races, and these genes are closely linked by coupling (Stavely, 1988). Since 1984, breeders have released 53 lines of beans resistant to all 55 rust races in the Beltsville collection (De Quattro, 1992). Newly released bean lines usually contain the identified resistant gene blocks $\underline{Up_2}$, which is effective against 23 races, and/or $\underline{Ur_3}$, which is effective against 29 races (Stavely et al., 1992). With the identification of additional groups of linked resistance genes, it should be possible to combine two or more independent linkage groups into bean

multilines that will then have two or more resistance genes to most races (Stavely, 1984c).

There are still many sources of specific resistance, but their stability is limited by pathogenic variability within geographical regions. They can be used more effectively by gene pyramiding, multiline, multiplasm, and regional deployment of genes to provide a geographically broad, longer lasting, and stable protection (Vargas, 1980).

2.3.1.2 Race Non-Specific Resistance. Non-specific resistance has also been observed and seems to be correlated with leaf epidermal characteristics. The surface topography of the leaf governs the ability of the germ tube to find a stomate (Heath, 1981). Shaik (1985b) showed that the mean number of pustules/$cm^2$ was positively correlated with the mean stomatal density on the adaxial leaf surface and negatively with the mean hair density on both surfaces.

The association of dense abaxial leaf pubescence with reduced infection intensity has been referred to as 'adult plant resistance.' This is expressed as significantly reduced uredinia size and density on the upper trifoliate leaves. While most of the resistance detected on primary leaves is race-specific, adult plant resistance is regarded as race-nonspecific (Mmbaga et al., 1991; Shaik, 1985; Shaik and Steadman, 1988). Adult plant resistance is found in Pompadour Checa and Jamaica Red which have dense straight hairs on their leaves' abaxial surface. The protection offered by the long hairs is greatest when the leaves have not expanded fully because the closeness of the hairs forms a thick mat over the surface on which the water droplets rest without making contact with the epidermis. The rust spores, being highly unwettable, rise to the water surface preventing germ tubes from making contact with the epidermis. Furthermore, the germ tubes tend to twine around the leaf hairs and get distracted from entering the leaf (Shaik and Steadman, 1988). Mmbaga and Steadman (1991a; 1991b), however, said that leaf pubescence cannot be the only factor involved in adult plant resistance

since sometimes glabrous genotypes also exhibited reduced uredinia density in the trifoliate leaves.

Vargas (1980) mentions other ways horizontal resistance is expressed such as reduced number of infections, decreased pustule size and spore production, and early telia formation. These are the symptoms of reduced fungal penetration and inhibition of fungal growth. Physical barriers are involved as well as wall-degrading enzymes (Heath, 1974) and toxic materials such as silicon-rich, electron-opaque deposits on and in mesophyll cell walls next to infection hyphae that prevent the first haustorium from breaching the affected wall (Heath, 1972).

Other factors that may also be present in non-specific resistance include a long incubation period, a slow rate of pustule development, and decreased pustule size in succeeding leaves (slow-rusting phenomenon) (Vargas, 1980). Several cultivars have been reported as 'slow-rusters' in several bean trials such as Tendercrop, Royal Red, Bush Romano, Astro, and Tidal Wave (Ballantyne, 1974; Meiners et al., 1975).

# 3. MATERIALS AND METHODS

## 3.1 IDENTIFICATION OF HAWAIIAN RACES OF BEAN RUST (*UROMYCES APPENDICULATUS* (PERS. EX PERS.) UNGER VAR. *APPENDICULATUS*)

### 3.1.1 Field Collection of Rust Isolates

Urediospore collections of bean rust were made in four locations in Hawaii (Table 2). Uredinia-bearing leaves of any age were sprinkled with a little water, placed in plastic bags to prevent dessication, and transported to the laboratory (Franje and Quebral, 1980). The leaves were shaken or scraped over a large sheet of paper to collect spores which were placed in a screw-capped vial and stored at -18°C (Stavely, 1983) in a refrigerator freezer until used for inoculations.

Table 2
Urediniospore Collections of Bean Rust in Hawaii

| Isolate | Location | Date | Cultivar Source | Planting Type |
|---|---|---|---|---|
| # 1 | Manoa, Oahu | 02/02/89 | Blue Lake | Community Garden |
| # 2 | Magoon Facility, Oahu | 03/15/89 | Pinto III | Research Planting |
| # 3 | Poamoho, Oahu | 03/18/89 | Poamoho | Bean Rust Test |
| # 4 | Manoa, Oahu | 03/24/89 | Kentucky Wonder | Community Garden |
| # 5 | Manoa, Oahu | 03/24/89 | Manoa Wonder | Community Garden |
| # 6 | Kula, Maui | 04/04/89 | Kentucky Wonder | Bean Rust Test |
| # 7 | Poamoho, Oahu | 08/28/89 | Hawaiian Wonder | Research Planting |

### 3.1.2 Spore Increase and Isolation from Single Pustules

The succeeding procedures, unless indicated otherwise, were all suggested by J.R. Stavely of the Beltsville Agricultural Research Center, ARS, USDA in Beltsville, Maryland (J.R. Stavely, personal communication).

Spores collected from the field were first increased in the greenhouse by inoculating fully susceptible bean cultivars such as Slenderette, Pinto 650, Pinto III or Early Gallatin. Field-collected spores were transferred from the screw-capped vials

using a small artist's brush moistened in an aqueous 0.1% Tween 20 solution to the lower surface of freshly opened unifoliate leaves of rust-free susceptible plants. A seed of any of the susceptible cultivars was sown in a 10 cm plastic pot containing a potting mixture of one part each of peat moss, perlite, and vermiculite. The recipient leaf was then water misted, and the whole plant enclosed in a plastic bag for 18-20 hours. The plastic bag was removed after the high humidity treatment and the plants were isolated from other rust-infected plants in the greenhouse to avoid contamination. After 12-14 days, urediospores from widely separated, single uredinia were transferred from the inoculated plants to other susceptible plants. The brush was repeatedly touched to the uredinium, then painted over the recipient leaf. The single uredinial isolations were repeated two or three times to obtain a population of urediospores that were uniformly virulent. The inoculum used for these isolations was highly diluted to produce pustules far apart from each other on a leaf to facilitate isolation of single pustules. Uninoculated check plants were included to ensure detection of any possible contamination (Bernier and Conner, 1982).

The resultant spores were collected 12-14 days after inoculation by tapping the leaves vigorously over an open (previously folded) large sheet of paper and collecting the spores in the crease. The spores were poured into a No. 42 sieve (45 apertures per linear inch) to separate them from debris. They were then poured into a small, screw-capped vial which was initially placed open over $CaCl_2$, a non-sulfurous dessicant, for 6-8 hours in a dessicator to remove excess humidity. They were then capped and stored at -18°C.

Each culture was increased every four months on isolated young bean plants of a susceptible bean cultivar to assure a ready supply of spores during the duration of this study.

### 3.1.3 Preparation of Inoculum Suspension for Testing

About 30 mg of spores were mixed with 50 ml of 0.01% Tween 20 in tap water in a 250 ml Erlenmeyer flask and stirred on a mix-stirrer at top speed for at least 2 min while adding another 50 ml of the Tween 20-water suspension to wet and disperse the spores. A hemacytometer was used to determine spore density which was standardized at 20,000 spores/ml (Menten and Filho, 1981)

### 3.1.4 Inoculation Method

The inoculum suspension was sprayed lightly for 0.5 sec on both surfaces of the unifoliate or primary leaves that were about one-third expanded with a Crown Spra-Tool modified with a plexiglass tube (Figure 4) to standardize the spray distance at 3.8 cm and spray diameter at 12 mm. The spray deposits were enough to wet the inoculated areas of the leaves but not to run off. Between different rust races, the equipment was sterilized by immersion in 10% sodium hypochlorite for about 30 min.

After the leaf surfaces dried, the plants were kept in a high-humidity chamber at 19°C for 18-24 hours, allowed to dry in the chamber, and then moved to a greenhouse. Plants were fertilized weekly with 15-30-15 liquid fertilizer.

### 3.1.5 Differential Bean Cultivars

Bean rust isolates were tested for race determination based on the reactions of 19 differential bean cultivars (Table 3). These cultivars were from a recommended set of 20 differential cultivars adopted during the Bean Rust Workshop in Puerto Rico (Stavely et al., 1983). The 20th cultivar (Mountaineer White Half Runner) was later dropped because it reacted to all races identically to Kentucky Wonder No. 780 (Stavely, 1984b).

The first six cultivars, U.S. #3, CSW 643, Pinto 650, K.W. 765, K.W. 780, and K.W. 814, were used by Harter and Zaumeyer (1941) in identifying 20 different rust

races. Early Gallatin was used by Christ and Groth (1982b) in their studies on rust isolates. The next five cultivars, A x S 37, Redlands Pioneer, Brown Beauty, Aurora, and NEP-2, were used by Ballantyne (1976) in her studies on rust resistance in eastern Australia. Olathe was released in Colorado by Wood and Keenan (1982) as a rust resistant Pinto. It derived its resistance from Golden Gate Wax and K.W. 765 (J.R. Stavely, personal communication). Ecuador 299, Mexico 235, Mexico 309, 51051, and Compuesto Negro Chimaltenango were added upon the suggestion of Pastor-Corrales from CIAT because they were consistently the most widely resistant entries in the 1975-1976 and 1977-1978 International Bean Rust Nurseries (IBRN).

Table 3
Differential Bean Cultivars

| Cultivar |
|---|
| Actopan x Sanilac (AxS) 37 |
| Aurora |
| Brown Beauty |
| California Small White (CSW) 643 |
| Compuesto Negro Chimaltenango (CNC) |
| Early Gallatin |
| Ecuador 299 |
| 51051 |
| Golden Gate Wax |
| Kentucky Wonder 814 |
| Kentucky Wonder 780 |
| Kentucky Wonder 765 |
| Mexico 309 |
| Mexico 235 |
| NEP-2 |
| Olathe |
| Pinto 650 |
| Redlands Pioneer |
| U.S.#3 |


Figure 4
Rust Inoculum Sprayer

Four seeds per cultivar were sown in 10 cm plastic pots in a potting mixture consisting of one part each of peat moss, perlite and vermiculite. The seeds were nicked opposite the hilum to increase uniformity in imbibition and germination. One pot of plants per cultivar was inoculated with each isolate in three trials, initiated 18 Aug., 3 Sept., and 25 Oct., 1989.

### 3.1.6 **Rust Grading Scale**

Disease readings were made 15 days after inoculation. The grading scale (Table 4) used here was developed in the 1983 Bean Rust Workshop (Stavely et al., 1983). It rates rust reactions into 6 grades on the basis of pustule size. This scale is based on Davison and Vaughan's (1963) scale which has 5 grades. Category #6 (> 0.8 mm) was added.

When making the readings, both leaf surfaces were examined. When several pustule grades were present on either surface, they were recorded in order of predominance, the most prevalent type being listed first, the least prevalent type last (Stavely et al., 1983). Stavely further rated reactions from immune (I) to very susceptible (VS) (Table 5, Figure 5).

## 3.2 PATTERNS OF INHERITANCE OF RESISTANCE TO THE HAWAIIAN RUST (*UROMYCES APPENDICULATUS* (PERS. EX PERS.) UNGER VAR. *APPENDICULATUS*) RACES

### 3.2.1 **Parental Crosses**

In order to study the inheritance of resistance to rust, crosses were made between various bean cultivars chosen for their rust reactions (Table 6).

Several seeds of each of these lines were planted in the Pope greenhouse of U.H. Manoa in 11.5-liter plastic pots containing one part each of peat moss, vermiculite and perlite. Plantings were made on 15 Dec. 1989 and 15 Jan. 1990, and crosses were

Table 4
Rust Grading Scale

| Grade | Symptoms on Primary Leaf 15 Days After Inoculation |
|---|---|
| 1 | Immune, no visible symptoms |
| 2 | Necrotic spots, but no sporulating pustules |
| 2 | Necrotic spots less than 300 $\mu$m in diameter |
| $2^+$ | Necrotic spots 300-1000 $\mu$m (1 mm) in diameter |
| $2^{++}$ | Necrotic spots 1-3 mm in diameter in diameter |
| $2^{+++}$ | Necrotic spots larger than 3 mm in diameter |
| 3 | Sporulating pustules less than 300 $\mu$m (0.3 mm) in diameter |
| 4 | Sporulating pustules 300-500 $\mu$m (0.3-0.5 mm) in diameter |
| 5 | Sporulating pustules 500-800 $\mu$m (0.5-0.8 mm) in diameter |
| 6 | Sporulating pustules larger than 800 $\mu$m (0.8 mm) in diameter |

Table 5
Rust Reaction Classes

| Grade or Grades | Reaction Class |
|---|---|
| 1 | **I** = Immune |
| 2, $2^+$, $2^{++}$, or $2^{+++}$ | **HR** = Highly Resistant |
| 3, 34, 23 or 32 | **R** = Resistant |
| 4 or 43 | **MR** = Moderately Resistant |
| 345, 45, 435, etc. | **MS** = Moderately Susceptible |
| 456, 546, 546, etc. | **S** = Susceptible |
| 6, 65, 654 | **VS** = Very Susceptible |

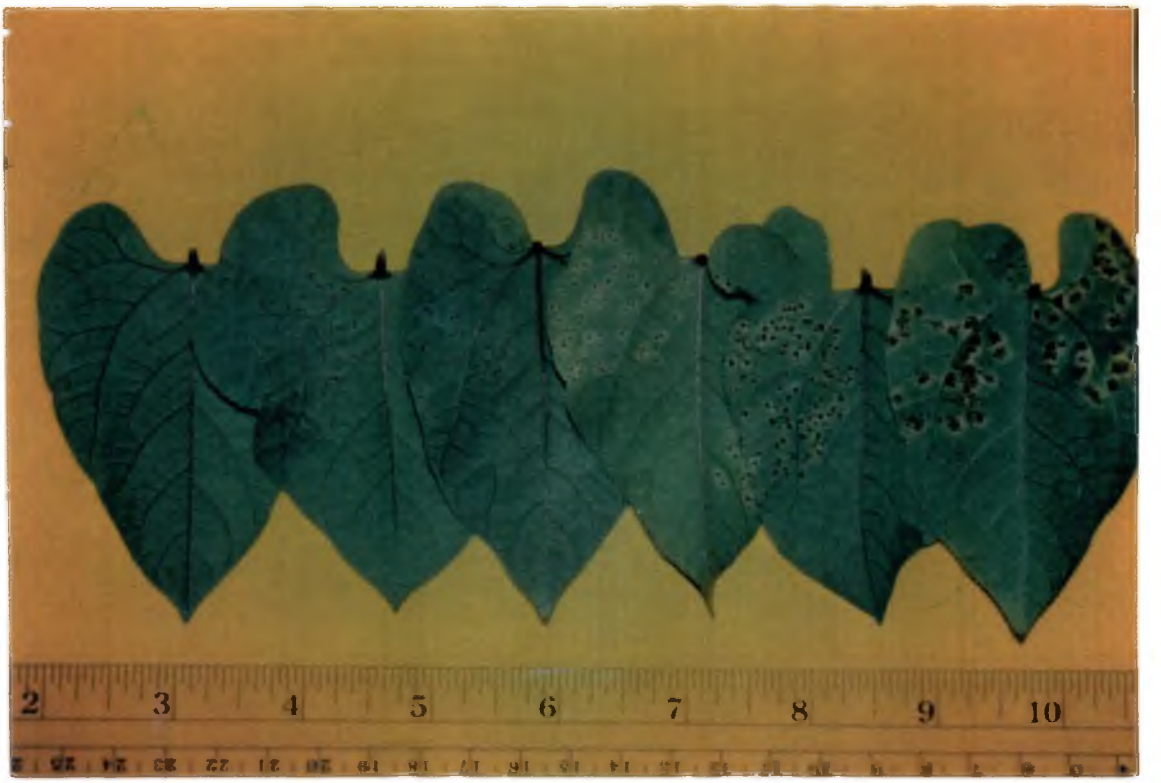


Figure 5
Bean Rust Reaction Classes

From left, immune (I), hypersensitive (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), and very susceptible (VS). (From Stavely, 1983)

Table 6
Parents Used in Crosses to Study Rust Resistance

| | |
|---|---|
| **Differential Cultivars** | |
| | **Resistant to all 4 Hawaiian isolates** |
| | Actopan x Sanilac 37 (AxS37) |
| | Compuesto Negro Chimaltenango (CNC) |
| | Ecuador 299 (Ec299) |
| | Mexico 235 |
| | Mexico 309 |
| | NEP-2 |
| | **Resistant to 2 Hawaiian isolates, susceptible to 2** |
| | California Small White 643 |
| | **Resistant to 1 Hawaiian isolate, susceptible to 3** |
| | Kentucky Wonder 765 |
| | **Susceptible to all 4 Hawaiian isolates** |
| | Aurora |
| | Golden Gate Wax |
| | Olathe |
| | Pinto 650 |
| **Hawaiian cultivars** | |
| | Hawaiian Wonder |
| | Poamoho |
| **'Slow Rusting'** according to Ballantyne (1974) | |
| | Astro |
| | Bush Romano |
| | Royal Red |
| | Tendercrop |
| | Tidal Wave |

made during the period January-March 1990 using the method described by Bliss (1980). Efforts were made to make all possible crosses among the parents, but some did not flower or set well, or did not flower at the same time. The combinations that were obtained and tested in the $F_2$ generation are shown in Figure 6.

3.2.1.1 Sterilization. Forceps and fingers were sterilized with alcohol whenever a new pollen source was used.

3.2.1.2 Preparation of the Female. The stigma is receptive at least two days before and one day after normal anthesis. Buds chosen on female parents were plump, showed color, and opened the next day. Emasculation was done by careful removal of the wings and keel of the flower. The 10 stamens were then removed carefully with forceps.

3.2.1.3 Preparation of the Pollen Source. Flowers were used as pollen sources the day they opened. A stigma with pollen on it was removed with forceps shortly after the flower opened in the morning and used immediately or placed in a plastic bag and kept in a refrigerator (about -18°C) for use in the afternoon.

3.2.1.4 Pollination. The pollen-carrying stigma was rubbed against the stigma of the female parent. The stigma from the pollen source was left in the female parent by hooking it through the style of the latter near the stigmatic surface. The standard was closed gently, and the bud was enclosed with cellophane tape which fell off as the petals withered. The pod started to develop 3-4 days after pollination. A tag carrying the appropriate information was placed around the pedicel of the female flower. All other flowers in the inflorescence containing the emasculated flower and all selfed pods were removed to avoid competition. The legumes resulting from pollinations were harvested when dry.

| FEMALE PARENT | MALE PARENT | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AxS37 | CNC | Ec299 | Mex235 | Mex309 | NEP-2 | CSW643 | Aurora | Olathe | P650 | HwnWon | Astro | BushRom | RoyalRed | TenderC. |
| AxS37 | | O | | | | | | | | | | O | O | | O |
| CNC | | | O | | O | | | O | | | | | | | O |
| Ec299 | O | O | | | | | O | O | O | O | | | | | O |
| Mex235 | | | | | | | | | O | | | | | | |
| Mex309 | | O | O | | | | O | O | O | | | | | | |
| NEP-2 | | | | | | | O | | O | | O | | | | |
| CSW643 | | O | O | O | | O | | O | O | O | O | | | O | O |
| KW765 | | | | | O | | | O | | | | | | | |
| Aurora | | O | O | | | | O | | O | O | | | | O | O |
| GGWax | | O | | | O | | | | | | | | | | |
| Olathe | | | | | | O | O | O | | | O | | | O | |
| P650 | | | O | | O | O | O | O | | | | | | O | |
| Poamoho | | | | | | O | O | | O | | | | | | |
| Astro | | | | | | | | | O | | | | | | |
| BushRom | | | O | | | | | | | | | | | O | |
| RoyalRed | | | O | | | | | O | O | O | | | | | |
| TenderC. | | | O | | | | O | | | | | | | | |
| TidalWave | | | | | | | | | | | | | | | O |

Figure 6
Combinations of Parental Lines That Produced $F_2$ Populations

### 3.2.2 Progeny Testing

The resulting $F_1$ seedlings were grown in the field (Poamoho Experimental Station) and allowed to self-fertilize to produce $F_2$ seeds. All $F_1$ plants grown were examined to distinguish actual $F_1$'s from inadvertent self-pollinations. Hypocotyl and flower color were examined on the $F_1$ plants when growing and seed coat color when harvested. All $F_1$ plants from which seed were saved were confirmed to be hybrids except for NEP-2 x CSW 643 and CSW 643 x Aurora, in which both parents had green hypocotyls, white flowers, and white seed coats. Both of these crosses did segregate for rust resistance in the $F_2$ and thus must have been $F_1$'s as expected.

$F_2$ seeds were collected from one $F_1$ plant of each cross to make up one $F_2$ family for each cross. The $F_2$ families were tested for rust reactions to all four pathotypes by using the confined spray method, which allowed the application of all four pathotypes on each plant. The two primary leaves were inoculated 6-8 days after seeding, that is, when the leaves were about 35-68% expanded, one pathotype on each side of each leaf. A small cut removing the apical end of one leaf blade was done to distinguish one primary leaf from the other. The trifoliate leaves were pinched off (Alten, 1983), and the apical meristem removed (Groth and Mogen, 1978) to increase the longevity of the primary leaves. No reciprocal differences were apparent in $F_2$ populations, so all data were combined. The results were evaluated with the chi-square test for goodness-of-fit (Ayala, 1982). Selected $F_2$ plants were transplanted to the field to produce $F_3$ progeny. The $F_3$ progeny from each $F_2$ plant were tested separately. In every rust test, Pinto 650 was used as a susceptible check.

# 4. RESULTS AND DISCUSSION

## 4.1 IDENTIFICATION OF HAWAIIAN RACES OF BEAN RUST (*UROMYCES APPENDICULATUS* (PERS. EX PERS.) UNGER VAR. *APPENDICULATUS*)

Of the seven rust isolates collected from different areas in Hawaii (Table 2), four were successfully propagated for further testing. Isolates #4, #5, and #7 did not produce a successful infection after inoculation in the greenhouse and were lost.

Thus, only four isolates were tested on the 19 differential bean cultivars (isolate #6 was relabelled as isolate #4). The results of these inoculations are given in Appendix A. The readings were highly consistent although some slight differences between pots or trials occurred. For several differentials with resistance to the Hawaiian isolates, 'fractional' readings (a difference in the degree of infection on the lower and upper sides of the leaf, the two readings being expressed in the form of a fraction) were obtained. The readings on the upper surface were always less virulent than those on the lower surface, so the lower surface readings were the ones used.

All four isolates were highly virulent (546 to 6) on U.S. #3, Pinto 650, K.W. 780, K.W. 814, Golden Gate Wax, Early Gallatin, Redlands Pioneer, Brown Beauty, Olathe, Aurora and 51051. All four isolates elicited the same degree of resistant reaction (3 or 34) from Mexico 309 and CNC. Necrotic reactions (2 or $2^+$) were observed from isolate #1 on Ecuador 299, Mexico 235, AxS 37, and NEP-2, and from isolate #2 on AxS 37. The necrotic reaction (2) was always associated with size 3, 4, and/or 5 pustules. The results of the differentials which actually differentiated between the isolates are shown in Table 7.

Isolates #1 (from Manoa) and #3 (from Poamoho) were quite similar, as were isolates #2 (from Magoon) and #4 (from Kula). Isolate #1 was less virulent on Ecuador 299, Mexico 235, AxS 37 and NEP-2 than isolate #3. This was based on the

presence of necrotic spots along with pustules from isolate #1 whereas from isolate #3, all infections formed pustules.

Table 7
Bean Rust Reactions on Differential Cultivars With Different Reactions for Different Isolates

| Differential Cultivar | Hawaiian Rust Isolate # 1 | # 2 | # 3 | # 4 |
|---|---|---|---|---|
| CSW 643 | 546[z] | 34,43 | 546 | 34,43 |
| K.W. 765 | 564,654 | 34 | 546,654 | 546,654 |
| Ecuador 299 | 234,243 | 3,34 | 43 | 43 |
| Mexico 235 | 243 | 3,34 | 34,43 | 3,43 |
| AxS 37 | 32+ | 2+3 | 34 | 34 |
| NEP-2 | 32+4 | 43 | 34 | 34,43 |

[z] 2 Necrotic spots, but no sporulating pustules
2 Necrotic spots less than 300 μm in diameter
2+ Necrotic spots 300-1000 μm (1 mm) in diameter
3 Sporulating pustules less than 300 μm (0.3 mm) in diameter
4 Sporulating pustules 300-500 μm (0.3-0.5 mm) in diameter
5 Sporulating pustules 500-800 μm (0.5-0.8 mm) in diameter
6 Sporulating pustules larger than 800 μm (0.8 mm) in diameter

Likewise, isolate #2 was less virulent than isolate #4. K.W. 765 had grade 3 or 4 pustules from isolate #2 but class 6 from isolate #4. AxS 37 had necrotic spots along with pustules from isolate #2 but all pustules from isolate #4. Isolate #4 also differed from the rest by its inability to grow very well in the greenhouse. On several dates, good pustule development was observed for the first three isolates but not for #4.

Thus, all four isolates that were tested turned out to be distinct. Since the four rust isolates were shown to be of different races, henceforth, they will be designated as H1 (for Hawaiian Race #1), H2, H3 and H4.

Since only one isolate was purified from each collection, there could have been more than one isolate present at some of the sites. Although the four isolates were not identical, they were quite similar and possibly originated from a common ancestor. It

is further possible that H4 evolved from H2 since both were similar except that H4 was highly virulent on K.W. 765 and more virulent on AxS 37. Likewise, H3 may have evolved from H1 since it was more virulent on Ecuador 299, Mexico 235, AxS 37 and NEP-2. In Hawaii, although the sexual stage of the pathogen does not occur, the bean rust fungus has a great potential to overcome resistance because of the high populations of urediniospores available to spread new mutations.

Comparisons with previously reported rust races showed that these Hawaiian races were different from the others. When the 19 differentials were used, the four Hawaiian rust races were very different from the 20 races (#38-57) reported by Stavely (1984b), the six from Minnesota and Wisconsin described by Groth and Shrum (1977), as well as the 9 Tanzanian races described by Mmbaga and Stavely (1988). When compared with the races identified by Harter and Zaumeyer (1941) using only U.S. #3, CSW 643, Pinto 650, K.W. 765, K.W. 780, and K.W. 814 as differentials, H1 and H3 were similar to their race 13, and H2 was closest to race 11 except that it was virulent on K.W. 780. Perhaps, when more races are evaluated with the same set of differential cultivars, these Hawaiian isolates may be found to also occur elsewhere.

## 4.2 PATTERNS OF INHERITANCE OF RESISTANCE TO THE HAWAIIAN RUST (*UROMYCES APPENDICULATUS* (PERS. EX PERS.) UNGER VAR. *APPENDICULATUS*) RACES

### 4.2.1 **Bean Rust Reactions of Parental Lines**

The lines used as parents to study the inheritance of resistance were the six differentials (Table 7), Mexico 309 and CNC, which were resistant (3 or 34) to all races, Aurora, G.G. Wax, Olathe and Pinto 650, which were susceptible (6, 65, 654, or 564) to all races, two Hawaiian cultivars, Hawaiian Wonder and Poamoho, and five cultivars reported by Ballantyne (1974) to be 'slow rusting.' Both Hawaiian cultivars

(Appendix B) and the slow rusting cultivars were all rated as susceptible (65 or 654) to all races, with one exception (Appendix C). Royal Red had a grade 34 reaction to H2.

The susceptible reaction of Hawaiian Wonder to all four races was surprising since this bean cultivar was developed for resistance to bean rust in Hawaii (Frazier and Hendrix, 1949). It has, however, been reported to have become susceptible in some locations (Hartmann, personal communication). Poamoho, bred for root-knot nematode resistance, also had no resistance to bean rust although Hawaiian Wonder was involved in its development (Hartmann, 1984).

The 'slow rusting' lines gave susceptible (65 or 654) reactions to all of the Hawaiian races with the exception of Royal Red which was resistant (34) to H2. These cultivars had been reported to show reduced pustule size and delayed sporulation, (Ballantyne, 1974), but they showed susceptible reactions to the Hawaiian races that were typical of those seen in other susceptible cultivars.

### 4.2.2 Accuracy of Individual $F_2$ Plant Designations

Individual plants in $F_2$ populations were evaluated for rust reactions by measuring the sizes of pustules as was done with the parents, according to the scale suggested in the 1983 Bean Rust Workshop (see Table 4, p. 34). The pustule sizes found were further classified into classes from immune to very susceptible as suggested by Stavely (see Table 5, p. 36). This classification was further modified by including in the highly resistant class all individuals that had any grade 2 spots even though they had pustules as well. Thus, the data in the Appendices D-G are given as classes I through VII, which are identical to Stavely's I through VS classes (Table 5) except for class II, which includes all individuals with any grade 2 spots even if most are pustules. $F_3$ families were grown from individual $F_2$ plants in the seven classes to test for accuracy of their classification.

4.2.2.1 Class I. Many of the $F_2$ populations showed a significant number of apparently immune (class I) individuals in addition to the resistant types that had been observed in the parents, especially when inoculated with race H2 (Appendix D-G). A sample of these class I plants were carried to the $F_3$ (Table 8). In the $F_3$, there were very few class I individuals, except in one family inoculated with race H2. Three families probably came from susceptible $F_2$ parents, but the remainder had mostly III, IV, and V reactions, and probably came from resistant $F_2$ plants. It is not known why the inoculations failed to 'take' on so many of the $F_2$ plants, since the susceptible Pinto 650 check plants were fully susceptible in all these tests. Perhaps, there is some environmental influence, because the most class I plants were obtained when inoculated with race H2, which seems closely related to race H4 which often did not grow in the greenhouse. Since the class I readings clearly seemed to be 'escapes,' they were excluded from the totals used to determine ratios.

4.2.2.2 Class II. In the $F_2$ progenies of Ecuador 299, Mexico 235, NEP-2, and AxS 37 inoculated with H1 and AxS 37 inoculated with H2, there were numerous plants with a class II reaction (necrosis, but also with pustule formation) (Appendix D and E), as had been seen in the parents. This type of reaction was rarely seen in progenies that did not involve these parents (Ec299 x Tendercrop in Appendix E). When individuals with this reaction were tested in the $F_3$ (Table 9), a high proportion of individuals had grade 2 spots, although there were some which did not. Sometimes, it was difficult to detect the very small ($<0.3$ mm) necrotic spots. $F_3$ testing of two $F_2$ class V 'escapes' showed a high proportion of the progeny with grade 2 spots. Therefore, any plant with grade 2 spots was called HR (hypersensitive), even though it also had spots that developed pustules.

Table 8
$F_3$ Segregations of Some Class I $F_2$ Plants

| $F_2$ Family | $F_2$Rxn. | Reaction Class[z] I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|---|
| **H1** | | | | | | | | | |
| Ec299CSW-56 | I | 0 | 23 | 4 | 2 | 0 | 0 | 0 | 29 |
| Ec299Ola-45 | I | 0 | 24 | 0 | 3 | 1 | 1 | 0 | 29 |
| NEPOla-25 | I | 0 | 8 | 1 | 2 | 3 | 1 | 0 | 15 |
| **H2** | | | | | | | | | |
| Ec299CSW-47 | I | 22 | 0 | 5 | 1 | 13 | 5 | 16 | 62 |
| Ec299BR-89 | I | 0 | 0 | 1 | 2 | 3 | 4 | 52 | 63 |
| Ec299Ola-48 | I | 0 | 0 | 26 | 3 | 2 | 0 | 0 | 31 |
| Ec299RR-6 | I | 0 | 0 | 0 | 1 | 0 | 10 | 2 | 13 |
| Ec299M309-19 | I | 0 | 0 | 8 | 19 | 1 | 0 | 1 | 29 |
| M309Aur-6 | I | 3 | 0 | 0 | 0 | 0 | 0 | 12 | 15 |
| OlaCSW-10 | I | 0 | 0 | 55 | 0 | 1 | 2 | 0 | 58 |
| **H3** | | | | | | | | | |
| Ec299CSW-24 | I | 0 | 0 | 3 | 12 | 0 | 0 | 0 | 15 |
| Ec299CSW-74 | I | 1 | 0 | 57 | 0 | 2 | 0 | 0 | 60 |
| Ec299TC-79 | I | 8 | 0 | 2 | 1 | 0 | 0 | 2 | 13 |
| M309Aur-88 | I | 2 | 0 | 32 | 8 | 1 | 0 | 20 | 63 |
| AurP650-53 | I | 3 | 0 | 0 | 0 | 0 | 0 | 27 | 30 |

[z] I = I of Stavely (no visible symptoms)
II = HR of Stavely but includes necrotic spots with size 3, 4 or 5 pustules
III = R of Stavely (3, 34)
IV = MR of Stavely (4, 43)
V = MS of Stavely (345, 45, 435, etc.)
VI = S of Stavely (456, 546, 564, etc.)
VII = VS of Stavely (6, 65, 654)

Table 9
$F_3$ Segregations of Some Class II $F_2$ Plants

| $F_2$ Family | $F_2$Rxn. | Reaction Class[z] I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|---|
| **H1** | | | | | | | | | |
| Ec299CSW-9 | II | 0 | 50 | 4 | 1 | 2 | 2 | 0 | 59 |
| Ec299CSW-48 | II | 1 | 51 | 5 | 0 | 2 | 2 | 2 | 67 |
| Ec299CSW-55 | V | 1 | 44 | 2 | 2 | 7 | 3 | 0 | 59 |
| Ec299P650-1 | V | 3 | 16 | 0 | 0 | 0 | 0 | 8 | 27 |

[z] I = I of Stavely (no visible symptoms)
II = HR of Stavely but includes necrotic spots with size 3, 4 or 5 pustules
III = R of Stavely (3, 34)
IV = MR of Stavely (4, 43)
V = MS of Stavely (345, 45, 435, etc.)
VI = S of Stavely (456, 546, 564, etc.)
VII = VS of Stavely (6, 65, 654)

4.2.2.3 Classes III, IV, and V. $F_2$ plants that were evaluated as being in classes III, IV, and V were also grown in the $F_3$ to test the accuracy of their classifications (Table 10). It seems that plants placed in any of these three classifications gave the same type of $F_3$ progeny. Therefore, plants which were originally classified as III, IV, or V were all combined and called R (resistant).

4.2.2.4 Classes VI and VII. When $F_3$ progeny of $F_2$ plants that had been classified as VI or VII were grown (Table 11), there was no difference between them. In some progenies, there were a few individuals which seemed to have some resistance, but these were probably cases where the pustules developed slowly for some reason. Therefore, all plants with VI or VII classifications were combined into one class called S (susceptible).

Table 10
$F_3$ Segregations of Some Class III, IV, or V $F_2$ Plants

| $F_2$ Family | $F_2$Rxn. | Reaction Class[z] I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|---|
| **H1** | | | | | | | | | |
| M309Aur-64 | III | 0 | 0 | 27 | 14 | 15 | 0 | 13 | 56 |
| M309Aur-72 | IV | 0 | 0 | 37 | 7 | 2 | 0 | 17 | 63 |
| M309Ola-2 | IV | 0 | 0 | 10 | 19 | 12 | 0 | 5 | 46 |
| M309Ola-8 | V | 1 | 0 | 10 | 12 | 5 | 0 | 3 | 31 |
| M309CSW-26 | IV | 0 | 0 | 12 | 8 | 1 | 6 | 3 | 30 |
| M309CSW-70 | III | 0 | 0 | 16 | 3 | 0 | 0 | 0 | 19 |
| **H2** | | | | | | | | | |
| Ec299CSW-4 | IV | 30 | 0 | 9 | 10 | 0 | 0 | 6 | 55 |
| Ec299CSW-55 | IV | 0 | 0 | 43 | 2 | 8 | 6 | 0 | 59 |
| Ec299CSW-74 | III | 3 | 3 | 50 | 1 | 2 | 1 | 0 | 60 |
| OlaNEP-30 | V | 0 | 0 | 9 | 2 | 0 | 0 | 0 | 11 |
| CSWP650-61 | IV | 3 | 0 | 1 | 5 | 5 | 0 | 2 | 16 |
| CSWP650-83 | V | 5 | 0 | 0 | 5 | 3 | 0 | 1 | 16 |
| AurCSW-68 | III | 1 | 0 | 3 | 9 | 43 | 4 | 0 | 63 |
| AurCSW-75 | IV | 0 | 0 | 6 | 10 | 40 | 6 | 0 | 62 |
| **H3** | | | | | | | | | |
| Ec299CSW-47 | IV | 8 | 0 | 36 | 8 | 9 | 0 | 2 | 63 |
| Ec299P650-1 | V | 0 | 0 | 9 | 5 | 2 | 2 | 8 | 26 |
| Ec299P650-2 | IV | 0 | 0 | 35 | 1 | 2 | 10 | 10 | 58 |
| Ec299BR-5 | III | 0 | 0 | 44 | 1 | 2 | 2 | 9 | 58 |
| Ec299BR-57 | IV | 4 | 0 | 43 | 3 | 0 | 2 | 4 | 56 |
| Ec299M309-19 | IV | 0 | 0 | 5 | 11 | 11 | 2 | 0 | 29 |
| M309CSW-4 | III | 0 | 0 | 2 | 7 | 8 | 0 | 11 | 28 |
| M309CSW-12 | IV | 0 | 0 | 19 | 6 | 0 | 0 | 0 | 25 |
| OlaNEP-25 | III | 0 | 0 | 3 | 1 | 8 | 2 | 1 | 15 |

[z] I = I of Stavely (no visible symptoms)
II = HR of Stavely but includes necrotic spots with size 3, 4 or 5 pustules
III = R of Stavely (3, 34)
IV = MR of Stavely (4, 43)
V = MS of Stavely (345, 45, 435, etc.)
VI = S of Stavely (456, 546, 564, etc.)
VII = VS of Stavely (6, 65, 654)

Table 11
$F_3$ Segregations of Class VI and VII $F_2$ Plants

| $F_2$ Family | $F_2$Rxn. | Reaction Class[z] I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|---|
| **H1** | | | | | | | | | |
| Ec299P650-7 | VII | 0 | 0 | 0 | 0 | 0 | 4 | 10 | 14 |
| Ec299P650-9 | VI | 0 | 0 | 0 | 0 | 0 | 2 | 14 | 16 |
| **H2** | | | | | | | | | |
| M309Aur-84 | VII | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 14 |
| M309CSW-55 | VII | 4 | 0 | 0 | 0 | 1 | 1 | 22 | 28 |
| OlaAur-57 | VII | 0 | 0 | 2 | 0 | 2 | 0 | 20 | 24 |
| OlaCSW-55 | VI | 0 | 0 | 0 | 0 | 6 | 8 | 2 | 16 |
| **H3** | | | | | | | | | |
| CSWP650-83 | VII | 0 | 0 | 0 | 0 | 3 | 8 | 4 | 15 |
| CSWP650-107 | VI | 0 | 0 | 0 | 1 | 3 | 9 | 1 | 14 |

[z] I = I of Stavely (no visible symptoms)
II = HR of Stavely but includes necrotic spots with size 3, 4 or 5 pustules
III = R of Stavely (3, 34)
IV = MR of Stavely (4, 43)
V = MS of Stavely (345, 45, 435, etc.)
VI = S of Stavely (456, 546, 564, etc.)
VII = VS of Stavely (6, 65, 654)

4.2.2.5 R Individuals in S x S Crosses. In crosses between two susceptible cultivars, although a majority of the $F_2$ plants showed susceptible reactions (classes VI and VII), some showed some resistance (class III, IV, or V). When the $F_3$ progeny of some of these class III, IV, or V individuals were tested (Table 12), they showed no resistance. Apparently, sometimes the pustules are slow to reach their maximum size.

4.2.2.6 Summary. On the basis of the $F_3$ families derived from $F_2$ plants, it was concluded that I (apparently immune) individuals were actually individuals on which inoculation did not take. All I individuals were therefore not included in determining ratios. Class II individuals all gave a large number of class II individuals in their progeny and were considered to show a hypersensitive reaction, even though they also developed pustules of various sizes. However, sometimes the hypersensitive spots would be overlooked and the individual misclassified according to the size of the

Table 12
$F_3$ Segregations of Some R $F_2$ Plants from Crosses
Between Two Susceptible Parents

| $F_2$ Family | $F_2$Rxn. | Reaction Class[z] I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|---|
| RRAur-71 | III | 0 | 0 | 0 | 0 | 1 | 12 | 2 | 15 |
| RRAur-69 | V | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 11 |
| CSWAur-55 | V | 0 | 0 | 0 | 0 | 0 | 57 | 6 | 63 |
| CSWAur-73 | IV | 1 | 0 | 0 | 0 | 2 | 50 | 7 | 60 |

[z] I = I of Stavely (no visible symptoms)
II = HR of Stavely but includes necrotic spots with size 3, 4 or 5 pustules
III = R of Stavely (3, 34)
IV = MR of Stavely (4, 43)
V = MS of Stavely (345, 45, 435, etc.)
VI = S of Stavely (456, 546, 564, etc.)
VII = VS of Stavely (6, 65, 654)

pustules. Class III, IV, and V $F_2$ individuals gave $F_3$ progeny that were mostly either class III, IV, or V and seemed similar to each other, so all three classes were combined into one resistant category. Class VI and VII $F_2$ individuals also gave $F_3$ populations which were not different from each other, so all such individuals were considered to be susceptible. Thus, there were escapes which showed no disease and were not included. There were cases where grade 2 spots were overlooked, and there were instances where pustules appaently developed slowly on a few plants, causing them to be misclassified in a lower class than they should have been. However, the number of the last two types of misclassification were small and probably did not affect the conclusions.

Thus, the $F_3$ evaluations have identified three types of rust reactions in these materials: HR (hypersensitive), R (resistant), and S (susceptible). When the six differential cultivars are reclassified this way, they still identify four Hawaiian races of bean rust (Table 7). The classifications of all the parents used to determine the inheritance of resistance are shown in Table 13.

Table 13
Rust Reactions of Parental Bean Lines

| Cultivar | H1 | H2 | H3 | H4 |
|---|---|---|---|---|
| **Differential Cultivars** | | | | |
| A x S 37 | HR | HR | R | R |
| Ecuador 299 | HR | R | R | R |
| Mexico 235 | HR | R | R | R |
| NEP-2 | HR | R | R | R |
| CSW 643 | S | R | S | R |
| K.W. 765 | S | R | S | S |
| **Other Resistant Parental Lines** | | | | |
| CNC | R | R | R | R |
| Mexico 309 | R | R | R | R |
| **Susceptible Parental Lines** | | | | |
| Aurora | S | S | S | S |
| G.G. Wax | S | S | S | S |
| Olathe | S | S | S | S |
| Pinto 650 | S | S | S | S |
| **Hawaiian Cultivars** | | | | |
| Hwn. Wonder | S | S | S | S |
| Poamoho | S | S | S | S |
| **'Slow Rusting' Cultivars** | | | | |
| Astro | S | S | S | S |
| Bush Romano | S | S | S | S |
| Royal Red | S | R | S | S |
| Tendercrop | S | S | S | S |
| Tidal Wave | S | S | S | S |

HR = Hypersensitive
R = Resistant
S = Susceptible

### 4.2.3 **Resistance to Hawaiian Race 1 (H1)**

The sources of resistance to H1 were Ecuador 299 (HR), AxS 37 (HR), NEP-2 (HR), Mexico 235 (HR), Mexico 309 (R), and CNC (R).

Ecuador 299. The $F_2$ segregations for resistance to H1 in crosses involving the hypersensitive (HR) Ecuador 299 are seen in Table 14.

Ecuador 299 was crossed with one HR parent, two R parents, and six S parents. In the cross with the other HR parent (AxS 37), no susceptible individuals were found, only HR and R individuals. It was previously shown that some HR spots may be overlooked, so these R plants should probably have been classified as HR and thus the $F_2$ would be considered uniform for this reaction.

All six $F_2$'s with susceptible parents (CSW 643, Pinto 650, Bush Romano, Tendercrop, Aurora, and Royal Red) had many HR individuals, a few R individuals, and some S individuals. If the R plants were included with the HR ones, as they probably should be, all these populations gave a good fit to a 3 hypersensitive: 1 susceptible ratio. The two crosses with R parents (CNC and Mexico 309) gave substantially more R individuals than the previous crosses. Both of these populations fit a 12 hypersensitive: 3 resistant: 1 susceptible ratio, which indicates that the gene for hypersensitive resistance in Ecuador 299 is epistatic to the gene for resistance in CNC and Mexico 309. Tentatively, the gene in Ecuador 299 is labelled $HR_1$, and the gene in CNC and Mexico 309 is labelled $R_1$ for hypersensitivity or resistance to H1, respectively.

Actopan x Sanilac 37 (AxS 37). In addition to its cross with Ecuador 299 (Table 14), AxS 37 was crossed with three S parents (Astro, Bush Romano, and Tendercrop) and one R parent (CNC). Table 15 shows that in the three crosses to susceptible lines, all showed a good fit to a 3:1 ratio, as did Ecuador 299 when crossed to susceptible parents. However, when AxS 37 was crossed to CNC, it did not give a good fit to a

Table 14
$F_2$ Segregations for Resistance to H1 in Crosses Involving Ecuador 299 (HR)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| Ec299xAxS37 | 18 | 6[z] | 0 | 24 | 100% Resistant | |
| Ec299xCNC | 58 | 22 | 9 | 89 | 4.96** (.10-.05) | 12:3:1 |
| Ec299xMex309 | 63 | 18 | 6 | 87 | 0.31** (.90-.75) | 12:3:1 |
| Ec299xCSW643 | 62 | 6[z] | 16 | 84 | 1.73** (.25-.10) | 3:1 |
| Ec299xP650 | 49 | 4[z] | 24 | 77 | 1.56** (.25-.10) | 3:1 |
| Ec299xBushRom | 93 | 6[z] | 37 | 136 | 0.35** (.75-.50) | 3:1 |
| Ec299xTenderC | 66 | 4[z] | 27 | 97 | 0.42** (.75-.50) | 3:1 |
| Ec299xAurora | 67 | 1[z] | 24 | 92 | 0.06** (.90-.75) | 3:1 |
| Ec299xRoy.Red | 20 | 5[z] | 6 | 31 | 0.53** (.50-.25) | 3:1 |

[z]Considered to be misclassified HR plants and included with HR for $X^2$ tests.

12:3:1 ratio, as did the cross of Ecuador 299 x CNC. There were too many susceptible individuals, and it is probable that many HR individuals have been misclassified as R individuals.

NEP-2. NEP-2 was also a parent with the hypersensitive resistance (HR) reaction. Results of crosses with NEP-2 are shown in Table 16. The lack of crosses with other HR parents prevented the determination of whether its HR gene is the same as the $HR_1$ of Ecuador 299. Two populations (with the susceptible Olathe and Pinto, 650) gave good fits to 3 HR: 1 S as expected with crosses to susceptible parents. The cross with Poamoho fit a 3:1 ratio, but not as well. However, the cross to the other

Table 15
F2 Segregations for Resistance to H1 in Crosses
Involving A x S 37 (HR)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| AxS37xAstro | 31 | 1[z] | 11 | 42 | 0.01** (.95-.90) | 3.1 |
| AxS37xBushRom | 28 | 1[z] | 11 | 40 | 0.13** (.75-.50) | 3:1 |
| AxS37xTenderC | 25 | 1[z] | 7 | 33 | 0.25** (.75-.50) | 3:1 |
| AxS37xCNC | 26 | 19 | 11 | 56 | 26.46[ns] (<0.01) | 12:3:1 |

[z]Considered to be misclassified HR plants and included with HR for $X^2$ tests.

Table 16
$F_2$ Segregations for Resistance to H1 in Crosses
Involving NEP-2 (HR)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| NEP-2xOlathe | 117 | 5[z] | 30 | 152 | 2.25** (.25-.10) | 3:1 |
| NEP-2xP650 | 18 | 1[z] | 7 | 26 | 0.27** (.90-.75) | 3:1 |
| NEP-2xPoam | 46 | 2[z] | 29 | 77 | 6.58* (.05-.01) | 3:1 |
| NEP-2xHwnWon. | 35 | 5[z] | 59 | 99 | 63.20[ns] (<0.01) | 3:1 |

[z]Considered to be misclassified HR plants and included with HR for $X^2$ tests.

Hawaiian cultivar, Hawaiian Wonder, gave a very different result, with many more susceptible individuals than expected. It is not possible to explain why there were less HR individuals in the crosses between NEP-2 and Poamoho and Hawaiian Wonder than with the equally susceptible Olathe and Pinto 650.

Mexico 235. This cultivar was also a source of hypersensitive resistance, but it was involved in only two crosses with small numbers of $F_2$ plants, namely, Mexico 235 x CSW 643 and Mexico 235 x Olathe. Both crosses fit the 3 hypersensitive resistant: 1 susceptible ratio (Table 17).

Table 17
$F_2$ Segregations for Resistance to H1 in Crosses Involving Mexico 235 (HR)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| Mex235xCSW643 | 25 | 2[z] | 7 | 34 | 0.35** (.75-.50) | 3:1 |
| Mex235xOlathe | 11 | 2[z] | 0 | 13 | 4.33* (.05-.01) | 3:1 |

[z]Considered to be misclassified HR plants and included with HR for $X^2$ tests.

Mexico 309. This cultivar was crossed with three susceptible parents (Aurora, CSW 643 and Olathe) and CNC (also R) (Table 18) as well as Ecuador 299 (HR) (Table 14). The $F_2$'s of the three crosses with susceptible parents all gave good fits to ratios of 3 R to 1 S. The cross with CNC, however, had a few susceptible individuals instead of being completely resistant, as would have been expected if both Mexico 309 and CNC had the same gene for resistance. The presence of these individuals could be explained by a second locus for resistance in CNC with a gene to be labelled $R_{1b}$. The gene in Mexico 309 then should be labelled $R_{1a}$.

Table 18
$F_2$ Segregations for Resistance to H1 in Crosses
Involving Mexico 309 (R)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| Mex309xAurora | 0 | 73 | 29 | 102 | 0.64** (.50-.25) | 3:1 |
| Mex309xCSW643 | 0 | 75 | 26 | 101 | 0.03** (.90-.75) | 3:1 |
| Mex309xOlathe | 0 | 30 | 13 | 43 | 0.63** (.50-.25) | 3:1 |
| Mex309xCNC | 0 | 153 | 3 | 156 | 4.98* (.05-.01) | 15:1 |

Compuesto Negro Chimaltenango (CNC). CNC, in addition to the cross with Ecuador 299 which gave a 12:3:1 ratio (Table 14) and with Mexico 309, which gave a 15:1 ratio (Table 18), was also crossed with the susceptible Aurora, CSW 643, Tendercrop, and G.G. Wax (Table 19). Only the cross with Aurora gave the expected ratio of 3 R to 1 S, as expected for one dominant gene ($R_{1b}$). The $F_2$'s of the other three crosses had more susceptible than resistant plants, suggesting a recessive gene for resistance might be segregating. However, it should be noted that the three populations which gave unexplained ratios had relatively small numbers of plants and thus may not have given accurate results.

Susceptible Lines. In crosses between two susceptible parents, the $F_2$ populations showed almost 100% susceptible individuals (Table 20). Although there were some individuals with resistance, these were probably susceptible reactions which were not expressed fully (see Table 12).

Summary. Thus, three possible genes for resistance to race H1 have been identified- $HR_1$ found in Ecuador 299 and AxS 37 and probably in NEP-2 and Mexico 235; $R_{1a}$ found in Mexico 309; and $R_{1b}$ found in CNC. All are dominant and give

Table 19
$F_2$ Segregations for Resistance to H1 in Crosses Involving Compuesto Negro Chimaltenango (CNC) (R)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| CNCxAurora | 0 | 81 | 29 | 110 | $0.11^{**}$ **(.75-.50)** | 3:1 |
| CNCxCSW643 | 0 | 10 | 20 | 30 | $27.78^{ns}$ **(<0.01)** | 3:1 |
| CNCxTenderC | 0 | 4 | 36 | 40 | $90.13^{ns}$ **(<0.01)** | 3:1 |
| CNCxG.G.Wax | 0 | 3 | 29 | 32 | $73.50^{ns}$ **(<0.01)** | 3:1 |

Table 20
$F_2$ Segregation for Resistance to H1 From Crosses Between Two Susceptible Parents

| Parents | HR | R | S | Total |
|---|---|---|---|---|
| Olathe x Aurora | 0 | 0 | 110 | 110 |
| Olathe x Poamoho | 0 | 1 | 205 | 206 |
| Olathe x HwnWonder | 0 | 0 | 48 | 48 |
| Olathe x RoyalRed | 0 | 0 | 107 | 107 |
| Olathe x Astro | 0 | 0 | 60 | 60 |
| CSW643 x P650 | 0 | 0 | 118 | 118 |
| CSW643 x Aurora | 0 | 2 | 101 | 103 |
| CSW643 x RoyalRed | 0 | 2 | 87 | 89 |
| CSW643 x TenderCrop | 0 | 0 | 158 | 158 |
| CSW643 x Poamoho | 0 | 0 | 136 | 136 |
| CSW643 x HwnWonder | 0 | 1 | 164 | 165 |
| RoyalRed x P650 | 0 | 3 | 83 | 86 |
| RoyalRed x Aurora | 0 | 12 | 59 | 79 |
| RoyalRed x BushRom | 0 | 0 | 37 | 37 |
| TenderC x BushRom | 0 | 0 | 57 | 57 |
| Aurora x K.W.765 | 0 | 0 | 46 | 46 |
| Aurora x P650 | 0 | 0 | 52 | 52 |
| Aurora x TenderCrop | 0 | 0 | 53 | 53 |

3:1 ratios in crosses with susceptible parents. $HR_1$ is epistatic to $R_{1a}$ and $R_{1b}$, giving 12:3:1 ratios in crosses where both are segregating. The evidence for the dominant genes $HR_1$ and $R_{1a}$ is quite strong, but is weaker for $R_{1b}$. More crosses need to be made to determine whether $R_{1a}$ and $R_{1b}$ are different genes.

### 4.2.4 Resistance to Hawaiian Race 2 (H2)

Data for resistance to H2 are not as extensive as for H1 because there were large numbers of seemingly immune plants, which were deleted from calculations of ratios (Appendix E). The parents with resistance to H2 are Ecuador 299 (R), AxS 37 (HR), NEP-2 (R), Mexico 235 (R), Mexico 309 (R), CNC (R), CSW 643 (R), Royal Red (R), and K.W. 765 (R).

Ecuador 299. Segregations in the $F_2$ of crosses involving the resistant cultivar Ecuador 299 are seen in Table 21. Crosses with susceptible cultivars like Pinto 650 and Aurora had $F_2$ segregations that were highly significant 3 resistant: 1 susceptible ratio indicating a one gene difference ($R_2$) between Ecuador 299 and these susceptible cultivars. Crosses with other susceptible cultivars like Bush Romano, Tendercrop, and Olathe yielded $F_2$ segregations that were significant at the 3:1, ratio but the number of individuals tested was low. When crossed with the resistant cultivars, CNC and Mexico 309, both the $F_2$'s segregated with a good fit to a 15 resistant: 1 susceptible ratio indicating the involvement of two resistance genes, one from each parent, interacting in a duplicate dominant epistasis. However, the crosses with CSW 643 and Royal Red, both also resistant, gave ratios of 3:1, not the 1:0 expected if both parents have the same gene for resistance nor the 15:1 expected if they have different genes. The cross with the hypersensitive resistant AxS 37 had only a few plants for testing, but some plants had necrotic spots (HR) as in the parent (Appendix E). Tentatively, the resistance gene in Ecuador 299 is designated as $R_{2a}$ and the gene in CNC and Mexico 309 is designated as $R_{2b}$.

Table 21
$F_2$ Segregations for Resistance to H2 in Crosses
Involving Ecuador 299 (R)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| Ec299xP650 | 0 | 49 | 18 | 67 | 0.12** (.75-.50) | 3:1 |
| Ec299xAurora | 0 | 125 | 49 | 174 | 0.93** (.50-.25) | 3:1 |
| Ec299xBushRom | 0 | 18 | 13 | 31 | 4.74* (.05-.01) | 3:1 |
| Ec299xTenderC | 0 | 39 | 7 | 46 | 2.35** (.25-.10) | 3:1 |
| Ec299xOlathe | 0 | 22 | 5 | 27 | 0.61** (.50-.25) | 3:1 |
| Ec299xMex309 | 0 | 100 | 5 | 105 | 0.40** (.75-.50) | 15:1 |
| Ec299xCNC | 0 | 176 | 15 | 191 | 0.84** (.50-.25) | 15:1 |
| Ec299xCSW643 | 0 | 54 | 12 | 66 | 1.64** (.25-.10) | 3:1 |
| Ec299xRoyalRed | 0 | 13 | 3 | 16 | 0.33** (.75-.50) | 3:1 |

AxS 37. This parent was the only cultivar which was HR to H2. It was crossed with several susceptible parents (Astro, Bush Romano, and Tendercrop) and with two resistant parents (Ecuador 299 and CNC), but there were very few individuals left to analyze after the class I were removed (Appendix E). Table 22 shows that the cross with Tendercrop fit a 3:1 ratio as expected, but the cross with CNC did not fit its expected 12:3:1 ratio. However, both types of reactions (HR and R) were present. Tentatively, the gene in AxS 37 is designated as $HR_2$.

NEP-2. The $F_2$ segregations of crosses involving NEP-2 are shown in Table 23. NEP-2 was crossed with two other resistant parents, Royal Red and CSW 643. The $F_2$'s of both of these crosses were entirely resistant, with no susceptible plants segregating. NEP-2 was also crossed with the susceptible parents Poamoho, Olathe, Pinto 650, and Hawaiian Wonder. The two populations with a large number of

Table 22
$F_2$ Segregations for Resistance to H2 in Crosses
Involving A x S 37 (HR)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| AxS37xTenderC | 11 | 9[z] | 3 | 23 | 1.75** (.25-.10) | 3:1 |
| AxS37xCNC | 13 | 21 | 7 | 41 | 62.91[ns] (<0.01) | 12:3:1 |

[z]Considered to be misclassified HR plants and included with HR for $X^2$ tests.

individuals both fit a 3:1 ratio as expected, as did one smaller population. However, the cross with Hawaiian Wonder did not fit a 3:1, but also did not have many plants evaluated since there were very many 'immune' plants in this population. Possibly, many of the plants on which the inoculation did not take would have been resistant, and then this population would have also fit a 3:1 ratio. It cannot be determined whether the gene for resistance in NEP-2 is the same as the one already found in Ecuador 299, CNC, and Mexico 309, since crosses between these lines were not made.

Mexico 235. This resistant (R) parent was crossed to one susceptible cultivar, Olathe, and to one R cultivar, CSW 643 (Appendix E). Neither cross had enough plants to analyze after the elimination of the class I (immune) plants.

Mexico 309. Mexico 309 has already been shown to segregate 15:1 when crossed with the resistant Ecuador 299 (Table 21). It also gave a 15:1 ratio when crossed with the resistant CNC (Table 24), but only gave a 3:1 ratio when crossed with the resistant CSW 643. It also gave a 3:1 ratio when crossed with the susceptible Aurora. It was also crossed with the susceptible Olathe, but only four plants were evaluated. It appears that the gene for resistance in Mexico 309 ($R_{2b}$) is different from both the gene in Ecuador 299 ($R_{2a}$) and the one in CNC, which is now designated $R_{2c}$.

Table 23
$F_2$ Segregations for Resistance to H2 in Crosses
Involving NEP-2 (R)

| Parents | HR | No. of Plants R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| NEP-2xRoyalRed | 0 | 32 | 0 | 32 | 100% Resistant | |
| NEP-2xCSW643 | 0 | 96 | 0 | 96 | 100% Resistant | |
| NEP-2xPoamoho | 0 | 161 | 33 | 194 | 6.60* (.05-.01) | 3:1 |
| NEP-2xOlathe | 0 | 88 | 24 | 112 | 0.76** (.50-.25) | 3:1 |
| NEP-2xPinto650 | 0 | 17 | 8 | 25 | 0.65** (.50-.25) | 3:1 |
| NEP-2xHwnWonder | 0 | 15 | 17 | 32 | 13.50ns (<0.01) | 3:1 |

Table 24
$F_2$ Segregations for Resistance to H2 in Crosses
Involving Mexico 309 (R)

| Parents | HR | No. of Plants R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| Mex309xAurora | 0 | 73 | 39 | 112 | 5.76* (.05-.01) | 3:1 |
| Mex309xCSW643 | 0 | 58 | 14 | 72 | 1.18** (.50-.25) | 3:1 |
| Mex309xCNC | 0 | 92 | 2 | 94 | 2.73** (.10-.05) | 15:1 |

Compuesto Negro Chimaltenango (CNC). CNC has already been shown to give 15:1 ratios when crossed with Ecuador 299 (Table 21) and Mexico 309 (Table 24). When crossed with AxS 37, both HR and R progeny were present, but the number of individuals was small and did not fit the expected 12:3:1 ratio. It was also crossed with the resistant CSW 643 and the susceptible Aurora, Tendercrop, and G.G. Wax

(Appendix E). The $F_2$ with CSW 643 did not fit either a 3:1 or 15:1 ratio, probably because there were very few plants (Table 25). The cross with Aurora did fit a 3:1 ratio as expected. There were too few plants in the other $F_2$'s to make any conclusions from them.

Table 25
$F_2$ Segregations for Resistance to H2 in Crosses
Involving Compuesto Negro Chimaltenango (CNC) (R)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| CNCxAurora | 0 | 52 | 7 | 59 | $5.43^{*}$ **(.05-.01)** | 3:1 |
| CNCxCSW643 | 0 | 17 | 12 | 29 | $61.08^{ns}$ **(<0.01)** | 15:1 |

California Small White 643. CSW 643 has already been shown to give all resistant progeny when crossed with NEP-2 (Table 23) and to segregate 3:1 in crosses with Mexico 309 (Table 24), Ecuador 299 (Table 21), and probably Mexico 235. The few plants of the population with CNC (Table 25) did not fit any expected ratio. Other crosses with CSW 643 are shown in Table 26. When crossed with the resistant Royal Red, it gave a good fit to a 3:1 ratio. It also gave a 3:1 ratio when crossed with the susceptible Poamoho, but did not give 3:1 ratios as expected in crosses with Hawaiian Wonder, Aurora, Tendercrop, and Pinto 650, all having an excess of susceptible individuals. On the other hand, when crossed with the susceptible Olathe, there were too many resistant individuals to fit a 3:1 ratio. Thus, CSW 643 gives variable results, sometimes seeming to transmit a gene for resistance, but other times not.

Royal Red. Royal Red gave all resistant progeny when crossed with NEP-2 (Table 23), as did CSW 643. However, when CSW 643 and Royal Red were crossed

Table 26
$F_2$ Segregations for Resistance to H2 in Crosses
Involving CSW 643 (R)

| Parents | HR | No. of Plants R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| CSW643xRoyalR | 0 | 137 | 51 | 188 | $0.45^{**}$ (.75-.50) | 3:1 |
| CSW643xPoamoho | 0 | 84 | 42 | 126 | $4.67^{*}$ (.05-.01) | 3:1 |
| CSW643xHwnWon | 0 | 26 | 52 | 78 | $72.22^{ns}$ (<0.01) | 3:1 |
| CSW643xAurora | 0 | 56 | 47 | 103 | $102.51^{ns}$ (<0.01) | 3:1 |
| CSW643xTenderC | 0 | 71 | 81 | 152 | $64.88^{ns}$ (<0.01) | 3:1 |
| CSW643xP650 | 0 | 50 | 37 | 87 | $14.35^{ns}$ (<0.01) | 3:1 |
| CSW643xOlathe | 0 | 120 | 20 | 140 | $8.57^{ns}$ (<0.01) | 3:1 |

(Table 26), the segregation was 3:1. Perhaps, the resistance gene from CSW 643 was not transmitted in this cross. When Royal Red was crossed with Ecuador 299 (Table 21), the ratio appeared to be 15:1, although only a small number of plants were evaluated. Royal Red was also crossed with four susceptible parents (Table 27). None of these fit the expected 3:1 ratio, but the numbers were small in all populations and may have been distorted by the large number of 'immune' plants that were deleted.

Kentucky Wonder 765 (K.W. 765). This resistant parent was involved in only one cross with a susceptible cultivar (Table 28). There was a good fit to the 3:1 ratio in the cross with Aurora to indicate the presence of a resistance gene ($R_2$).

Susceptible Lines. Table 29 shows the $F_2$ segregations of crosses between two susceptible parents. Although there were some R individuals in two of the crosses, when some were tested in the $F_3$, all were susceptible.

Table 27
$F_2$ Segregations for Resistance to H2 in Crosses
Involving Royal Red (R)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| RoyalRxAurora | 0 | 19 | 17 | 36 | $9.48^{ns}$ **(<0.01)** | 3:1 |
| RoyalRxBushRom | 0 | 7 | 20 | 27 | $34.68^{ns}$ **(<0.01)** | 3:1 |
| RoyalRxP650 | 0 | 35 | 32 | 67 | $18.51^{ns}$ **(<0.01)** | 3:1 |
| RoyalRxOlathe | 0 | 17 | 21 | 38 | $18.56^{ns}$ **(<0.01)** | 3:1 |

Table 28
$F_2$ Segregations for Resistance to H2 in Crosses
Involving Kentucky Wonder 765 (R)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| KW765xAurora | 0 | 35 | 7 | 42 | $1.56^{**}$ **(.25-.10)** | 3:1 |

Table 29
$F_2$ Segregation for Resistance to H2 From Crosses
Between Two Susceptible Parents

| Parents | HR | R | S | Total |
|---|---|---|---|---|
| Olathe x Aurora | 0 | 0 | 33 | 33 |
| Olathe x Poamoho | 0 | 25 | 98 | 123 |
| Olathe x Astro | 0 | 0 | 14 | 14 |
| Aurora x P650 | 0 | 0 | 47 | 47 |
| Aurora x TenderC | 0 | 0 | 50 | 50 |
| TenderCxTidalWave | 0 | 0 | 18 | 18 |
| TenderCxBushRomano | 0 | 5 | 24 | 29 |

Summary. It is more difficult to interpret the results for testing for resistance to H2 than it was for H1. There were many more 'immune' individuals which were escapes and many ratios which did not fit the expected, quite likely because of the large number of 'immune' reactions interfering with resistant ones. The gene ($HR_2$) in AxS 37 was shown to be transmitted. The resistance genes in Ecuador 299, Mexico 309, NEP-2, CSW 643, Royal Red, and K.W. 765 all seemed to be transmitted at least some of the time. The genes in NEP-2, CSW 643, and Royal Red may be the same, but Ecuador 299, Mexico 309, and CNC all seem to have different genes ($R_{2a}$, $R_{2b}$, and $R_{3c}$).

4.2.5 **Resistance to Hawaiian Race 3 (H3)**

The number of immune $F_2$'s in tests with H3 were not as numerous as in the tests with H2 (Appendix F). There were no HR reactions except for one individual from Ec299 x Pinto 650. The resistant (R) parents were Ecuador 299, AxS 37, NEP-2, Mexico 235, Mexico 309, and CNC.

Ecuador 299. Ecuador 299 was crossed with three resistant and seven susceptible parents (Table 30). There were no susceptible segregates in the cross with the resistant Mexico 309, indicating that both parents have the same gene for resistance ($R_{3a}$). The cross with the resistant CNC segregated 15:1, indicating that these two parents have different genes for resistance to this race. The gene in CNC is tentatively designated $R_{3b}$. No susceptible individuals were obtained from the cross with the resistant AxS 37, but the number of plants was too small to make any conclusions. All seven crosses with susceptible parents fit 3:1 ratios, showing that in these crosses there is one gene difference between the two parents.

Actopan x Sanilac 37 (AxS 37). Besides its cross with Ecuador 299, AxS 37 was also crossed with the resistant CNC and three susceptible parents (Table 31). Although numbers were small, it seemed to segregate 15:1 with CNC and 3:1 with the other

parents. This indicates that AxS 37 has one gene for resistance which is different from the gene in CNC, much like was found for Ecuador 299. Possibly, AxS 37 and Ecuador 299 have the same gene for resistance, since no susceptible segregates were found in the small number of individuals in their $F_2$.

NEP-2. NEP-2 was crossed with five susceptible parents (Table 32). The cross with Olathe segregated 3:1 as expected, as did the cross with Pinto 650, although the numbers in the latter were small. The crosses with two Hawaiian cultivars, Poamoho and Hawaiian Wonder, did not fit a 3:1 ratio, but had many more susceptible plants than expected. When the cross between NEP-2 and Hawaiian Wonder was tested for resistance to H1, it showed a very similar distribution, with more susceptible

Table 30
$F_2$ Segregations for Resistance to H3 in Crosses
Involving Ecuador 299 (R)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| Ec299xMex309 | 0 | 111 | 0 | 111 | 100% Resistant | |
| Ec299xCNC | 0 | 191 | 17 | 208 | 1.31** (.50-.25) | 15:1 |
| Ec299xAxS 37 | 0 | 10 | 0 | 10 | | |
| Ec299xCSW643 | 0 | 79 | 14 | 93 | 4.91* (.05-.01) | 3:1 |
| Ec299xP650 | 0 | 111 | 44 | 155 | 0.95** (.50-.25) | 3:1 |
| Ec299xBushRom | 0 | 116 | 31 | 147 | 1.20** (.50-.25) | 3:1 |
| Ec299xTenderC | 0 | 75 | 15 | 90 | 3.33** (.10-.25) | 3:1 |
| Ec299xAurora | 0 | 135 | 51 | 186 | 0.58** (.50-.25) | 3:1 |
| Ec299xOlathe | 0 | 102 | 29 | 131 | 0.57** (.50-.25) | 3:1 |
| Ec299xRoyalRed | 0 | 37 | 8 | 45 | 1.25** (.50-.25) | 3:1 |

Table 31
$F_2$ Segregations for Resistance to H3 in Crosses
Involving A x S 37 (R)

| Parents | HR | No. of Plants R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| AxS37xCNC | 0 | 41 | 7 | 48 | 5.69* (.05-.01) | 15:1 |
| AxS37xAstro | 0 | 29 | 10 | 39 | 0.01** (.90-.75) | 3:1 |
| AxS37xBushRom | 0 | 25 | 9 | 34 | 0.04** (.90-.75) | 3:1 |
| AxS37xTenderC | 0 | 24 | 8 | 32 | 0.00** (0.99) | 3:1 |

Table 32
$F_2$ Segregations for Resistance to H3 in
Crosses Involving NEP-2 (R)

| Parents | HR | No. of Plants R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| NEP-2xOlathe | 0 | 120 | 38 | 158 | 0.08** (.90-.75) | 3:1 |
| NEP-2xP650 | 0 | 19 | 7 | 26 | 0.05** (.90-.75) | 3:1 |
| NEP-2xPoamoho | 0 | 83 | 108 | 191 | 101.36ns (<0.01) | 3:1 |
| NEP-2xHwn Won. | 0 | 42 | 59 | 101 | 60.15ns (<0.01) | 3:1 |

individuals than expected. Since each $F_2$ came from a single $F_1$ plant, there cannot have been a mixture of genotypes in the $F_2$.

Mexico 235. There were two crosses with susceptible parents and both fit a 3:1 ratio although the number of plants was small for each cross (Table 33). This indicates the presence of an $R_3$ gene. There were no crosses made with other resistant parents.

Table 33
$F_2$ Segregations for Resistance to H3 in
Crosses Involving Mexico 235 (R)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| Mex235xOlathe | 0 | 10 | 0 | 10 | 3.33** (.10-.05) | 3:1 |
| Mex235xCSW643 | 0 | 17 | 5 | 22 | 0.06** (.90-.75) | 3:1 |

Mexico 309. The cross between Mexico 309 and Ecuador 299 did not segregate (Table 30), indicating that both have the same resistance gene. Table 34 shows the results of crosses with the susceptible parents, Aurora, Olathe and CSW 643, and with a resistant parent, CNC. Such results confirm that Mexico 309 has a gene for resistance to H3 and that the gene is different from the $R_{3b}$ gene present in CNC. Thus, Mexico 309 has the $R_{3a}$ gene found in Ecuador 299.

Compuesto Negro Chimaltenango (CNC). CNC has already been shown to have a different gene for resistance ($R_{3b}$) than is found in Ecuador 299 (Table 30), AxS 37 (Table 31), and Mexico 309 (Table 34), which are probably all the same ($R_{3a}$). CNC was also crossed with four susceptible parents (Table 35). Only with Aurora did it give the expected 3:1 ratio. The other three crosses did not fit a 3:1 ratio, but the number of individuals was small in all three populations.

Table 34
$F_2$ Segregations for Resistance to H3 in Crosses
Involving Mexico 309 (R)

| Parents | HR | No. of Plants R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| Mex309xAurora | 0 | 130 | 42 | 172 | $0.03^{**}$ (.90-.75) | 3:1 |
| Mex309xCSW643 | 0 | 128 | 47 | 175 | $0.32^{**}$ (.75-.50) | 3:1 |
| Mex309xOlathe | 0 | 92 | 47 | 139 | $5.76^{*}$ (.05-.01) | 3:1 |
| Mex309xCNC | 0 | 125 | 3 | 128 | $3.83^{**}$ (.10-.05) | 15:1 |

Table 35
$F_2$ Segregations for Resistance to H3 in Crosses
Involving Compuesto Negro Chimaltenango (CNC) (R)

| Parents | HR | No. of Plants R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| CNCxAurora | 0 | 84 | 24 | 108 | $0.44^{**}$ (.75-.50) | 3:1 |
| CNCxCSW643 | 0 | 12 | 18 | 30 | $19.6^{ns}$ (<0.01) | 3:1 |
| CNCxTenderC | 0 | 3 | 37 | 40 | $97.2^{ns}$ (<0.01) | 3:1 |
| CNCxG.G.Wax | 0 | 1 | 23 | 24 | $16.0^{ns}$ (<0.01) | 3:1 |

Susceptible Lines. The $F_2$ segregations of crosses among susceptible parents are shown in Table 36. Again, all the progeny were susceptible with only a few exceptions.

Table 36
$F_2$ Segregation for Resistance to H3 From Crosses Between Two Susceptible Parents

| Parents | HR | R | S | Total |
|---|---|---|---|---|
| Olathe x Aurora | 0 | 0 | 110 | 110 |
| Olathe x Poamoho | 0 | 0 | 206 | 206 |
| Olathe x RoyalRed | 0 | 0 | 107 | 107 |
| Olathe x Astro | 0 | 0 | 60 | 60 |
| CSW643 x P650 | 0 | 0 | 207 | 207 |
| CSW643 x Aurora | 0 | 7 | 96 | 103 |
| CSW643 x RoyalRed | 0 | 12 | 177 | 189 |
| CSW643 x TenderC | 0 | 0 | 158 | 158 |
| CSW643 x Poamoho | 0 | 6 | 130 | 136 |
| CSW643 x Hwn.Won. | 0 | 0 | 167 | 167 |
| CSW643 x Olathe | 0 | 8 | 85 | 93 |
| RoyalRed x P650 | 0 | 3 | 83 | 86 |
| TenderC x BushRom | 0 | 0 | 57 | 57 |
| Aurora x P650 | 0 | 0 | 50 | 50 |
| Aurora x TenderC | 0 | 0 | 54 | 54 |

Summary. Two resistance genes, designated as $R_{3a}$ and $R_{3b}$, for H3 were identified. Both genes are dominant and give 3:1 ratios in crosses with susceptible parents. $R_{3a}$ is found in Ecuador 299 and Mexico 309 and probably AxS 37 and has proven to be different from $R_{3b}$ which is found in CNC. The $R_3$ gene found in NEP-2 and Mexico 235 could not be tested for identity with $R_{3a}$ or $R_{3b}$ since there were no crosses with Ecuador 299, Mexico 309, AxS 37, or CNC.

### 4.2.6 Resistance to Hawaiian Race 4 (H4)

Although the initial testing of the differentials' reaction to this rust race yielded satisfactory results, subsequent spore increase and testing of the $F_2$ frequently did not work. Successful infection occurred only in a few $F_2$'s, and no $F_3$ tests were obtained.

Ecuador 299, AxS 37, NEP-2, Mexico 235, Mexico 309, CNC, and CSW 643 were resistant (R) to H4. Only some of these cultivars are considered in the succeeding sections.

Ecuador 299. Ecuador 299 was crossed with 3 resistant and 3 susceptible parents (Table 37). No susceptible segregates were obtained from the cross with Mexico 309, indicating that both parents have the same gene for resistance. The crosses with both CNC and CSW 643 segregated 15:1, indicating that they have a different gene from Ecuador 299. All three crosses with susceptible parents gave 3:1 ratios as expected, confirming that Ecuador 299 has a dominant gene for resistance to H4. The gene in Ecuador 299 is tentatively designated $R_{4a}$ and the gene in CNC and CSW 643 $R_{4b}$.

Table 37
$F_2$ Segregations for Resistance to H4 in Crosses
Involving Ecuador 299 (R)

| Parents | HR | No. of Plants R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| Ec299xMex309 | 0 | 90 | 0 | 90 | 100% Resistant | |
| Ec299xCNC | 0 | 89 | 2 | 91 | 2.55** (.25-.10) | 15:1 |
| Ec299xCSW643 | 0 | 65 | 5 | 70 | 0.09** (.90-.75) | 15:1 |
| Ec299xP650 | 0 | 52 | 18 | 70 | 0.02** (.90-.75) | 3:1 |
| Ec299xAurora | 0 | 65 | 27 | 92 | 0.93** (.50-.25) | 3:1 |
| Ec299xOlathe | 0 | 44 | 9 | 53 | 1.81** (.25-.10) | 3:1 |

NEP-2. NEP-2 was only crossed with two susceptible parents (Table 38). The cross with Olathe showed a good fit to a 3:1 ratio expected in a cross between a resistant and susceptible parent, but the cross with Poamoho did not. There were many more susceptible individuals that expected, just as happened when the same cross was

tested for H1 and H3 (when tested with H2, however, it fit a 3:1 ratio and even had more resistant plants than expected).

Table 38
$F_2$ Segregations for Resistance to H4 in
Crosses Involving NEP-2 (R)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| NEP-2xOlathe | 0 | 65 | 26 | 91 | $0.62^{**}$ **(.50-.25)** | 3:1 |
| NEP-2xPoamoho | 0 | 21 | 57 | 78 | $96.15^{ns}$ **(<0.01)** | 3:1 |

Mexico 235. A single cross was made with a susceptible parent, Olathe (Table 39). Such a cross had a good fit to a 3:1 ratio to indicate the presence of a resistance gene ($R_4$) in Mexico 235.

Table 39
$F_2$ Segregations for Resistance to H4 in
Crosses Involving Mexico 235 (R)

| Parents | No. of Plants HR | R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| Mex235xOlathe | 0 | 40 | 10 | 50 | $0.67^{**}$ **(.50-.25)** | 3:1 |

Mexico 309. Mexico 309 has already been shown to show no segregation when crossed with Ecuador 299 (Table 37). It was also crossed with the resistant parents CNC and CSW 643 and the susceptible parents Aurora and Olathe (Table 40). It gave 15:1 ratios with CNC and CSW 643, just as these two parents did with Ecuador 299, confirming that the gene in Ecuador 299 and Mexico 309 is different than the one in

CNC and CSW 643. The ratios in both crosses with susceptible parents were 3:1 as expected.

Table 40
$F_2$ Segregations for Resistance to H4 in Crosses
Involving Mexico 309 (R)

| Parents | HR | No. of Plants R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| Mex309xCNC | 0 | 85 | 4 | 89 | 0.47** (.50-.25) | 15:1 |
| Mex309xCSW643 | 0 | 78 | 10 | 88 | 3.93* (.05-.01) | 15:1 |
| Mex309xAurora | 0 | 69 | 26 | 95 | 0.28** (.75-.50) | 3:1 |
| Mex309xOlathe | 0 | 67 | 24 | 91 | 0.09** (.90-.75) | 3:1 |

Compuesto Negro Chimaltenango (CNC). Besides Ecuador 299 and Mexico 309, CNC was also crossed with CSW 643 (Table 41). Although CNC has been shown to have a different gene than Ecuador 299 and Mexico 309, it seems to also have a different gene than CSW 643 because it gave a 15:1 ratio when crossed with it. The gene in CSW 643 would then be designated $R_{4c}$.

Table 41
$F_2$ Segregations for Resistance to H4 in Crosses
Between Compuesto Negro Chimaltenango (CNC) (R) and CSW 643 (R)

| Parents | HR | No. of Plants R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| CNCxCSW643 | 0 | 67 | 4 | 71 | 0.05** (.90-.75) | 15:1 |

California Small White (CSW) 643. Besides its crosses with the resistant parents which indicate it has a gene for resistance ($R_{4c}$) different from all the others, CSW 643

was crossed with four susceptible parents (Table 42). Only the cross with Olathe gave a 3:1 ratio as expected. The crosses with Pinto 650, Royal Red, and Hawaiian Wonder all had many more susceptible individuals than expected.

Table 42
$F_2$ Segregations for Resistance to H4 in Crosses
Involving CSW 643 (R)

| Parents | HR | No. of Plants R | S | Total | Chi Square | Expected Ratio |
|---|---|---|---|---|---|---|
| CSW643xOlathe | 0 | 74 | 18 | 92 | $1.45^{**}$ **(.25-.10)** | 3:1 |
| CSW643xP650 | 0 | 35 | 53 | 88 | $58.24^{ns}$ **(<0.01)** | 3:1 |
| CSW643xRoyalR | 0 | 74 | 53 | 127 | $18.96^{ns}$ **(<0.01)** | 3:1 |
| CSW643xHwn.Won | 0 | 10 | 50 | 60 | $108.89^{ns}$ **(<0.01)** | 3:1 |

Susceptible Lines. There were three crosses between susceptible parents (Table 43) tested for their $F_2$ segregation for resistance to H4. There were more apparently resistant individuals found than in the other races, perhaps, an indication of the difficulty in getting H4 to grow in the greenhouse.

Table 43
$F_2$ Segregation for Resistance to H4 From Crosses
Between Two Susceptible Parents

| Parents | HR | R | S | Total |
|---|---|---|---|---|
| Olathe x Aurora | 0 | 34 | 30 | 64 |
| Olathe x Poamoho | 0 | 17 | 73 | 90 |
| RoyalRed x BushRom | 0 | 2 | 24 | 26 |

Summary. Thus, there seem to be three dominant genes for resistance to H4- $R_{4a}$ in Ecuador 299 and Mexico 309, $R_{4b}$ in CNC, and $R_{4c}$ in CSW 643. As with other

races, Ecuador 299, Mexico 309, and CNC nearly always gave 3:1 ratios as expected when crossed with susceptible parents, but NEP-2 and CSW 643 often did not, giving many more susceptible plants than expected.

### 4.2.7 Genotypes of Resistant Parents

Table 44 shows the resistance genes that were deduced to be present among the resistant parental lines. In these materials, there were two types of resistance detected-hypersensitive (HR) in which necrotic spots developed with some pustule formation, and resistance (R) in which only small pustules developed. Ratios observed were 1:0 when both parents apparently had the same gene, 15:1 when two resistant parents had different genes, 12:3:1 when one parent was hypersensitive and the other resistant, and 3:1 when either a hypersensitive or resistant parent was crossed with a susceptible one. In addition, there were progenies which did not fit these ratios for unknown reasons.

Table 44
Genotypes of Resistant Parents

| Cultivar | H1 | H2 | H3 | H4 |
|---|---|---|---|---|
| Ecuador 299 | $HR_1$ | $R_{2a}$ | $R_{3a}$ | $R_{4a}$ |
| AxS 37 | $HR_1$ | $HR_2$ | $R_{3a}$ | $R_{4x}$ |
| NEP-2 | $HR_1$ | $R_{2x}$[z] | $R_{3x}$ | $R_{4x}$ |
| Mexico 235 | $HR_1$ | | $R_{3x}$ | $R_{4x}$ |
| Mexico 309 | $R_{1a}$ | $R_{2b}$ | $R_{3a}$ | $R_{4a}$ |
| CNC | $R_{1b}$ | $R_{2c}$ | $R_{3b}$ | $R_{4b}$ |
| CSW 643 | | $R_{2x}$ | | $R_{4c}$ |
| K.W.765 | | $R_{2x}$ | | |
| Royal Red | | $R_{2x}$ | | |

[z] $R_{2x}$, $R_{3x}$, $R_{4x}$ mean identity with other R genes not tested.

Of the 45 $F_2$ families tested for rust segregations, 18 families showed at least one poor fit to the expected ratios when inoculated with the four fungal races. Crosses that had problematic segregations for one race also had segregations that did not fit in the other races. However, in all cases, the reason the segregations did not fit the expected

ratios was that there was an excess of susceptible individuals. Thus, the reason for the poor fits was not a lack of infection, as is often experienced in testing for resistance, but too much infection.

It was noticed that, of the 18 families giving poor fits, 17 were in the first of the three plantings of $F_1$'s in the field. In the first planting, 17 of 24 families had poor fits, in the second planting, none of 6, and in the third planting, one of 15. Thus, it appears that at the time the first planting flowered, there may have been a large amount of natural outcrossing which did not occur when the other two plantings flowered. Such instances of outcrossing interspersed with periods when there is none have been noticed at this farm previously (Hartmann, personal communication), but have occurred so seldomly that precautions to prevent it are not considered necessary.

Thus, the probable reason for so many abnormal ratios, and especially involving CSW 643 and Royal Red (for which the $F_1$'s were in the first planting), is that the $F_1$'s were crossed naturally in the field with other susceptible plants instead of being selfed as expected.

### 4.2.8 Linkage Analysis

When $F_2$ data were being taken, it was noticed that most individuals that were resistant to one race were also resistant to other races. Therefore, the $F_2$ data were tested for linkage between the genes for resistance to the four different races (Table 45). Twenty-four crosses between a parent resistant to all four races and one resistant to none showed linkage values ranging from 0 to 22.92%, with most values less than 10%. This suggests that all the genes for resistance evaluated in this study are linked together as a block.

These results show that even the different genes for resistance to one race alone must be linked to each other also, since, for example, if gene $R_{2a}$ in Ecuador 299 is closely linked to gene $R_{3a}$ which is closely linked to $R_{2b}$ in Mexico 309, then the $R_{2b}$

gene must also be closely linked to $R_{2a}$. This contradicts the previous conclusions that the different genes for resistance to the same race are independently inherited with 15:1 ratios when both parents have the R gene or 12:3:1 ratios when one parent has a HR gene.

The crosses between two parents both resistant to the same race were therefore reexamined to see if the results could be explained by assuming close linkage with a small amount of crossing over to give the few susceptible individuals (Table 46). The linkage values calculated from these crosses varied from 1.92 to 19.64%, very similar to the range of values calculated for the crosses between resistant and susceptible parents.

Therefore, this linked complex of genes includes not only genes for resistance to different races, but different genes for resistance to the same race, and includes a total of at least 12 genes. Complexes of genes conferring resistance to many races have previously been reported by Stavely et al. (1992). They reported that such a complex occurred in Mexico 235, one of the parents used here. However, this complex cannot be the same as Stavely's because they reported that the same complex was present in Aurora, which did not have any resistance in the present study. Stavely and Steinke (1985) also reported 17 dominant genes that are linked in coupling in Mexico 309 but since the four Hawaiian rust races were shown to be different from other races, the four resistance genes in Mexico 309 are different from the reported 17 genes but could be parts of the linked complex of dominant resistance genes in the cultivar.

Table 45
Linkage Data

| Parental Cross | Loci | Phenotypic Classes[z] a | b | c | d | total | p[y] |
|---|---|---|---|---|---|---|---|
| Ec299xP650 | $HR_1R_{2a}$ | 41 | 0 | 6 | 17 | 64 | 9.38 |
| | $HR_1R_{3a}$ | 50 | 1 | 5 | 18 | 74 | 8.11 |
| | $R_{2a}R_{3a}$ | 45 | 4 | 1 | 17 | 67 | 7.46 |
| | $R_{3a}R_{4a}$ | 46 | 3 | 6 | 15 | 70 | 12.86 |
| Ec299xBushRom | $HR_1R_{2a}$ | 13 | 0 | 4 | 12 | 29 | 13.79 |
| | $HR_1R_{3a}$ | 98 | 0 | 6 | 30 | 134 | 4.48 |
| | $R_{2a}R_{3a}$ | 18 | 1 | 0 | 12 | 31 | 3.23 |
| Ec299xTenderC | $HR_1R_{2a}$ | 30 | 0 | 11 | 7 | 48 | 22.92 |
| | $HR_1R_{3a}$ | 45 | 1 | 9 | 10 | 65 | 15.38 |
| | $R_{2a}R_{3a}$ | 36 | 6 | 0 | 7 | 49 | 12.24 |
| Ec299xAurora | $HR_1R_{2a}$ | 51 | 0 | 0 | 18 | 69 | 0 |
| | $HR_1R_{3a}$ | 50 | 1 | 0 | 19 | 70 | 1.43 |
| | $HR_1R_{4a}$ | 49 | 1 | 0 | 20 | 70 | 1.43 |
| | $R_{2a}R_{3a}$ | 106 | 2 | 0 | 41 | 149 | 1.34 |
| | $R_{2a}R_{4a}$ | 51 | 0 | 0 | 20 | 71 | 0 |
| | $R_{3a}R_{4a}$ | 48 | 0 | 0 | 20 | 68 | 0 |
| Ec299xOlathe | $R_{2a}R_{3a}$ | 14 | 8 | 0 | 5 | 37 | 21.62 |
| | $R_{3a}R_{4a}$ | 42 | 0 | 2 | 8 | 52 | 3.85 |
| Mex309xAurora | $R_{1a}R_{2b}$ | 13 | 0 | 0 | 17 | 30 | 0 |
| | $R_{2b}R_{3a}$ | 72 | 0 | 3 | 36 | 111 | 2.70 |
| | $R_{3a}R_{4a}$ | 69 | 1 | 0 | 22 | 92 | 1.09 |
| Mex309xOlathe | $R_{3a}R_{4a}$ | 59 | 0 | 9 | 24 | 92 | 9.78 |
| CNCxAurora | $R_{1b}R_{2c}$ | 49 | 0 | 3 | 7 | 59 | 5.08 |
| | $R_{1b}R_{3b}$ | 83 | 0 | 0 | 10 | 93 | 0 |
| | $R_{2c}R_{3b}$ | 21 | 2 | 0 | 3 | 26 | 7.69 |

[z] a : resistant to both races
b : resistant to 1st race, susceptible to 2nd race
c : susceptible to 1st race, resistant to 2nd race
d : susceptible to both races
[y] p : crossover percentage

Table 46
Linkage Among Resistance Genes for a Single Race

| Parents | Loci | HR | R | S | Total | $p^y$ |
|---|---|---|---|---|---|---|
| **H1** | | | | | | |
| Ec299xCNC | $HR_1R_{1b}$ | 58 | 22 | 9 | 89 | 10.11 |
| Ec299xMex309 | $HR_1R_{1a}$ | 63 | 18 | 6 | 87 | 6.90 |
| AxS37xCNC | $HR_1R_{1b}$ | 26 | 19 | 11 | 56 | 19.64 |
| Mex309xCNC | $R_{1a}R_{1b}$ | 0 | 153 | 3 | 156 | 1.92 |
| **H2** | | | | | | |
| Ec299xMex309 | $R_{2a}R_{2b}$ | 0 | 100 | 5 | 105 | 4.76 |
| Ec299xCNC | $R_{2a}R_{2c}$ | 0 | 176 | 15 | 191 | 7.85 |
| **H3** | | | | | | |
| CNCxMex309 | $R_{3b}R_{3a}$ | 0 | 125 | 3 | 128 | 2.34 |
| CNCxEc299 | $R_{3b}R_{3a}$ | 0 | 191 | 17 | 208 | 8.17 |
| **H4** | | | | | | |
| CNCxMex309 | $R_{4b}R_{4a}$ | 0 | 85 | 4 | 89 | 4.49 |
| CNCxCSW643 | $R_{4b}R_{4c}$ | 0 | 67 | 4 | 71 | 5.63 |
| Ec299xCSW643 | $R_{4a}R_{4c}$ | 0 | 65 | 5 | 70 | 7.14 |
| Mex309xCSW643 | $R_{4a}R_{4c}$ | 0 | 78 | 10 | 88 | 11.36 |

HR = Hypersensitive
R = Resistant
S = Susceptible
$^y p$ = crossover percentage

# 5. SUMMARY AND CONCLUSIONS

Four races of bean rust (*Uromyces appendiculatus* (Pers. ex Pers.) Unger var. *appendiculatus*) have been identified from several isolates collected from different locations in Hawaii using a set of nineteen differential bean cultivars that has been adopted as standard by the International Bean Rust Workshop. The four races were actually differentiated by six of the nineteen differentials. The closest similarity occurred between H1 (Manoa) and H3 (Poamoho) despite being collected from separate areas. They could be differentiated from each other by means of the necrotic reactions with pustules caused by H1 on Ecuador 299, AxS 37, Mexico 235, and NEP-2 which were not observed from H3. H2 (Magoon) caused necrosis with pustule formation on AxS 37 but formed only pustules on the other cultivars. This Magoon race could also be distinguished by the resistance of K.W. 765. H4 (Maui) was identified by the resistance of CSW 643 and the susceptibility of K.W. 765. Comparisons with published reactions of other races of bean rust showed that these Hawaiian races of bean rust are different from other previously reported races.

Resistance to these Hawaiian races of bean rust in some of the differentials appears to be conditioned by a series of several dominant genes, one per race, that are tightly linked by coupling and is expressed either as the formation of hypersensitive spots along with small pustules (HR gene) or as small pustules without hypersensitive spots (R gene). The HR gene appears to be epistatic to the R gene. There are suggestions of a recessive resistance gene in some cultivars, but this needs further investigation because a number of progenies did not give the expected ratios.

Testing of the progeny of crosses between resistant and susceptible lines and between resistant lines indicated that for H1, Ecuador 299, AxS 37, Mexico 235, and NEP-2 are sources of an HR gene while CNC and Mexico 309 have an R gene. Only

AxS 37 has an HR gene for H2 while Ecuador 299, Mexico 235, NEP-2, CNC, Mexico 309, CSW 643, K.W. 765, and Royal Red have R genes for this race. All these cultivars with the exception of CSW 643 and K.W. 765 have an R gene for H3. The same cultivars with the exception of K.W. 765 also have an R gene for H4. All 12 genes identified in these cultivars are closely linked into a gene block or series with low crossing over between genes for resistance to different races as well as genes for resistance to the same race.

There are numerous reports in the literature of single genes conferring resistance to bean rust. There are also reports of complexes of genes conferring resistance to many races of the bean rust. In this study, 12 different genes for resistance were identified in a total of 9 parents resistant to one or more of the four races identified. None were inherited independently. The fact that all 12 genes identified were all linked in one complex suggests that this might be a general situation in this species. If this is correct, as more and more genes are added to the block for resistance to these and other races, they will be transmitted together and it should be easier to accumulate genes for resistance without losing them to segregation when they are incorporated into commercial cultivars. Thus, cultivars with a block of genes for resistance to many different races should be able to withstand attack by new races of the pathogen much longer than a cultivar that has one or a very few different genes for resistance.

## Appendix A
## Rust Reactions of Differential Bean Cultivars to Four Hawaiian Rust Isolates in Three Trials
## (4 pots/entry unless indicated otherwise)

| Differential Cultivar | Trial I (08/11/89) | | | | Trial II (08/25/89) | | | | Trial III (10/18/89) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | #1 | #2 | #3 | #4 | #1 | #2 | #3 | #4 | #1 | #2 | #3 | #4 |
| U.S. #3 | 654[z] | 546/65[y] | 654 | 654,65 | 654 | 546/65[x] | 654 | 65 | 654,65 | 65 | 65 | 65 |
| CSW 643 | 546 | 43 | 546 | 43 | 546 | 43 | 546 | 43 | 546 | 34 | 546 | 34/43 |
| Pinto 650 | 65 | 6 | 654 | 654 | 654,65 | 654,65 | 65 | 65 | 654,65 | 65 | 654 | 654,65 |
| K.W. 765 | 654 | 34/43 | 654 | 654 | 654 | 34/43 | 546 | 546 | 564 | 34[w] | 654 | 546 |
| K.W. 780 | 654 | 65 | 654 | 654 | 654 | | 654,65 | 654 | 654 | 65 | 654 | 654 |
| K.W. 814 | 654 | 654 | 654 | 654 | 654 | 546 | 654 | 654 | 654 | 65 | 564 | 654 |
| G.G. Wax | 564 | 65 | 65 | 654 | 6,56 | 65 | 65 | 654 | 65 | 65,56 | 65 | |
| Early Gallatin | 564 | 6,65,56 | 564 | 65 | 564 | 564 | 564 | | 564 | 564 | 564 | 65,654 |
| Redlands Pioneer | 654 | 65[x] | 65,654 | 65 | 654[w] | 654[x] | 564 | 65[z] | 65,654 | 65 | 65 | 65 |
| Ecuador 299 | 243/2+3 | 34/2+3 | 43/2+3 | 43/2+34 | 243/2+3 | 3/2+3 | 43/2+3 | 43/2+3 | 234/2+3 | 34/2+3 | 43/2+3 | 43/2+3 |
| Mexico 235 | 243/2+3 | 34/2+3 | 43/2+3 | 43/2+3 | 243/2+3 | 3/2+3 | 43/2+3 | 43/2+3 | 243/2+3 | 34/2+3 | 3/2+3 | 3/2+3 |
| Mexico 309 | 3/2+3 | 3/2+3 | 3/2+3 | 34/2+3 | 3/2+3 | 3/2+3 | 3/2+3 | 3/2+3 | 3/2+3 | 3/2+3 | 3/2+3 | 3/2+3 |
| Brown Beauty | 564 | 654[w] | | 65,654 | 65,564 | 564 | 564 | 65 | 564 | 56 | | 65 |
| Olathe | 654 | | 654 | 65,654 | 654 | 65[x] | 654 | 546 | 654,564 | 65 | 654,564 | 546 |
| A x S 37 | 32+/2+32+ | 34/2+3 | 34 | 34 | 32+2+ | 3/2+ | 34/32+ | 34 | 32+/2+32+ | 3/2+ | | 34 |
| NEP-2 | 32+4/34 | 43 | 3/34 | 43 | 32+4/43 | 43 | 34/43 | 43 | 32+4/43 | 43 | 34/43 | 34/43 |
| Aurora | 654 | 65 | 654 | 654 | 654 | 564 | 654 | 65,654 | 654,564 | 65 | 654 | 654 |
| 51051 | 564 | 564 | 654 | 654 | 654 | 654 | 654 | 654 | 654,564 | 654 | 654 | 654 |
| CNC | 34/2+3 | 34/2+3 | 34/2+3 | 34 | 34/2+3 | 3/2 | 34/2+3 | 34/2+3 | 3/2+3 | 34/2+3 | 3/2+3 | 34/2+3 |

[w] 3 pots
[x] 2 pots
[y] Indicates abaxial reading as numerator and adaxial reading as denominator
[z] Pustule size graded 1-6 according to Stavely et al. (1983). See Table 4, p. 34.

Appendix B
Rust Reactions of Hawaiian Bean Cultivars to Four Hawaiian Rust Races in Three Trials

| Differential Cultivar | Trial I (08/11/89) | | | | Trial II (08/25/89) | | | | Trial III (10/18/89) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H1 | H2 | H3 | H4 | H1 | H2 | H3 | H4 | H1 | H2 | H3 | H4 |
| Hawaiian Wonder | 654[z] | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 |
| Poamoho | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 |

[z] Pustule size graded 1-6 according to Stavely et al. (1983). See Table 4, p. 34.

Appendix C
Rust Reactions of 'Slow Rusting' Bean Cultivars to Four Hawaiian Rust Races in Three Trials

| Differential Cultivar | Trial I (01/14/90) | | | | Trial II (08/18/91) | | | | Trial III (02/18/92) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H1 | H2 | H3 | H4 | H1 | H2 | H3 | H4 | H1 | H2 | H3 | H4 |
| Astro | 65[z] | 65 | 654 | 654 | 65 | 654 | 654 | 654 | 65 | 654 | 654 | 654 |
| Bush Romano | 65 | 654 | 65 | 65 | 65 | 654 | 65 | 65 | 65 | 654 | 65 | 65 |
| Royal Red | 654 | 34 | 654 | 654 | 654 | 34 | 654 | 654 | 654 | 34 | 654 | 654 |
| Tendercrop | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 | 654 |
| Tidal Wave | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 |

[z] Pustule size graded 1-6 according to Stavely et al. (1983). See Table 4, p. 34.

Appendix D
$F_2$ Segregation for Resistance to H1

| Parents | Reaction Class[z] I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|
| Ec299xCSW643 | 9 | 62 | 3 | 1 | 2 | 1 | 15 | 94 |
| Ec299xPinto650 | 3 | 49 | 0 | 1 | 3 | 7 | 17 | 80 |
| Ec299xBush Romano | 16 | 93 | 0 | 3 | 3 | 8 | 29 | 152 |
| Ec299xTenderCrop | 14 | 66 | 0 | 0 | 4 | 15 | 12 | 111 |
| Ec299xAurora | 0 | 67 | 0 | 0 | 1 | 13 | 11 | 92 |
| Ec299xOlathe | 56 | 0 | 0 | 2 | 0 | 1 | 1 | 60 |
| Ec299xRoyal Red | 51 | 20 | 1 | 2 | 2 | 0 | 6 | 82 |
| Ec299xCNC | 0 | 58 | 15 | 1 | 6 | 8 | 1 | 89 |
| Ec299xMexico309 | 0 | 63 | 14 | 4 | 0 | 3 | 3 | 87 |
| Ec299xAxS37 | 12 | 18 | 2 | 4 | 0 | 0 | 0 | 36 |
| AxS37xAstro | 5 | 31 | 0 | 1 | 0 | 3 | 8 | 48 |
| AxS37xBushRomano | 0 | 28 | 0 | 1 | 0 | 1 | 10 | 40 |
| AxS37xTenderCrop | 3 | 25 | 1 | 0 | 0 | 0 | 7 | 36 |
| AxS37xCNC | 3 | 26 | 0 | 11 | 8 | 5 | 6 | 59 |
| Mex235xOlathe | 4 | 11 | 1 | 1 | 0 | 0 | 0 | 17 |
| Mex235xCSW643 | 8 | 25 | 1 | 0 | 1 | 0 | 7 | 42 |
| NEP-2xOlathe | 21 | 117 | 3 | 1 | 1 | 17 | 13 | 174 |
| NEP-2xPoamoho | 0 | 46 | 2 | 0 | 0 | 20 | 9 | 77 |
| NEP-2xHwnWonder | 6 | 35 | 3 | 1 | 1 | 6 | 53 | 105 |
| NEP-2xPinto650 | 0 | 18 | 1 | 0 | 0 | 0 | 7 | 26 |
| Mex309xAurora | 1 | 0 | 57 | 16 | 0 | 2 | 27 | 103 |
| Mex309xOlathe | 0 | 0 | 3 | 13 | 14 | 2 | 11 | 43 |
| Mex309xCNC | 24 | 0 | 117 | 25 | 11 | 2 | 1 | 180 |
| Mex309xCSW643 | 0 | 0 | 54 | 19 | 2 | 2 | 24 | 101 |
| CNCxAurora | 1 | 0 | 28 | 53 | 0 | 19 | 10 | 111 |
| CNCxCSW643 | 1 | 0 | 1 | 1 | 8 | 11 | 9 | 31 |
| CNCxTenderCrop | 0 | 0 | 0 | 0 | 4 | 12 | 24 | 40 |
| CNCxG.G.Wax | 0 | 0 | 0 | 1 | 2 | 3 | 26 | 32 |
| OlathexAurora | 0 | 0 | 0 | 0 | 1 | 0 | 109 | 110 |
| OlathexCSW643 | 0 | 0 | 4 | 8 | 25 | 22 | 13 | 72 |
| OlathexPoamoho | 0 | 0 | 0 | 1 | 7 | 59 | 139 | 206 |
| OlathexHwnWonder | 2 | 0 | 0 | 0 | 5 | 2 | 41 | 50 |
| OlathexRoyal Red | 0 | 0 | 0 | 0 | 0 | 15 | 92 | 107 |
| OlathexAstro | 0 | 0 | 0 | 0 | 0 | 2 | 58 | 60 |
| CSW643xPinto650 | 0 | 0 | 0 | 0 | 5 | 20 | 93 | 118 |
| CSW643xAurora | 0 | 0 | 0 | 2 | 25 | 35 | 41 | 103 |
| CSW643xRoyalRed | 0 | 0 | 1 | 1 | 23 | 14 | 50 | 89 |
| CSW643xTenderCrop | 0 | 0 | 0 | 0 | 11 | 39 | 108 | 158 |
| CSW643xPoamoho | 0 | 0 | 0 | 0 | 8 | 25 | 103 | 136 |
| CSW643xHwnWonder | 2 | 0 | 0 | 1 | 36 | 15 | 113 | 167 |
| RoyalRedxPinto650 | 0 | 0 | 0 | 3 | 5 | 6 | 72 | 86 |
| RoyalRedxAurora | 8 | 0 | 3 | 9 | 15 | 10 | 34 | 79 |
| RoyalRedxBushRomano | 0 | 0 | 0 | 0 | 2 | 1 | 34 | 37 |
| TenderCxBushRomano | 0 | 0 | 0 | 0 | 0 | 9 | 48 | 57 |
| KW765xAurora | 0 | 0 | 0 | 0 | 8 | 17 | 21 | 46 |

Appendix D (Continued)
$F_2$ Segregation for Resistance to H1

| Parents | Reaction Class[z] I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|
| AuroraxPinto650 | 1 | 0 | 0 | 0 | 0 | 0 | 52 | 53 |
| AuroraxTenderCrop | 1 | 0 | 0 | 0 | 0 | 7 | 46 | 54 |

[z] I = I of Stavely (no visible symptoms)
II = HR of Stavely but includes necrotic spots with size 3, 4 or 5 pustules
III = R of Stavely (3, 34)
IV = MR of Stavely (4, 43)
V = MS of Stavely (345, 45, 435, etc.)
VI = S of Stavely (456, 546, 564, etc.)
VII = VS of Stavely (6, 65, 654)

Appendix E
$F_2$ Segregation for Resistance to H2

| Parents | Reaction Class[z] I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|
| Ec299xCSW643 | 46 | 0 | 2 | 30 | 6 | 1 | 9 | 94 |
| Ec299xPinto650 | 13 | 0 | 29 | 16 | 4 | 3 | 15 | 80 |
| Ec299xBush Romano | 121 | 0 | 10 | 7 | 1 | 0 | 13 | 152 |
| Ec299xTenderCrop | 62 | 3 | 26 | 9 | 4 | 1 | 6 | 111 |
| Ec299xAurora | 15 | 0 | 93 | 27 | 5 | 18 | 31 | 189 |
| Ec299xOlathe | 33 | 0 | 12 | 8 | 2 | 0 | 5 | 60 |
| Ec299xRoyal Red | 66 | 0 | 8 | 3 | 2 | 1 | 2 | 82 |
| Ec299xCNC | 20 | 0 | 127 | 41 | 8 | 14 | 1 | 211 |
| Ec299xMexico309 | 30 | 0 | 94 | 5 | 1 | 2 | 3 | 135 |
| Ec299xAxS37 | 25 | 5 | 4 | 2 | 0 | 0 | 0 | 36 |
| AxS37xCNC | 18 | 13 | 6 | 11 | 4 | 1 | 6 | 59 |
| AxS37xAstro | 34 | 7 | 1 | 1 | 0 | 0 | 4 | 48 |
| AxS37xBushRomano | 32 | 4 | 0 | 0 | 0 | 0 | 4 | 40 |
| AxS37xTenderCrop | 13 | 11 | 6 | 3 | 0 | 1 | 2 | 36 |
| Mex235xOlathe | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 17 |
| Mex235xCSW643 | 26 | 0 | 8 | 2 | 2 | 0 | 4 | 42 |
| NEP-2xPoamoho | 0 | 0 | 63 | 45 | 53 | 25 | 8 | 194 |
| NEP-2xHwnWonder | 73 | 0 | 4 | 6 | 5 | 0 | 17 | 105 |
| NEP-2xRoyal Red | 6 | 0 | 32 | 0 | 0 | 0 | 0 | 38 |
| NEP-2xPinto650 | 1 | 0 | 6 | 5 | 6 | 1 | 7 | 26 |
| NEP-2xCSW643 | 1 | 0 | 71 | 19 | 6 | 0 | 0 | 97 |
| NEP-2xOlathe | 62 | 0 | 52 | 12 | 24 | 15 | 9 | 174 |
| Mex309xAurora | 84 | 0 | 62 | 11 | 0 | 2 | 37 | 196 |
| Mex309xOlathe | 39 | 0 | 0 | 0 | 1 | 0 | 3 | 43 |
| Mex309xCSW643 | 20 | 0 | 43 | 14 | 1 | 2 | 12 | 92 |
| Mex309xCNC | 86 | 0 | 85 | 3 | 4 | 1 | 1 | 180 |
| CNCxAurora | 52 | 0 | 24 | 23 | 5 | 0 | 7 | 111 |
| CNCxCSW643 | 2 | 0 | 1 | 3 | 13 | 6 | 6 | 31 |
| CNCxTenderCrop | 1 | 0 | 0 | 0 | 1 | 11 | 27 | 40 |
| CNCxG.G.Wax | 22 | 0 | 0 | 0 | 0 | 1 | 9 | 32 |
| CSW643xOlathe | 121 | 0 | 19 | 47 | 54 | 9 | 2 | 252 |
| CSW643xPinto650 | 1 | 0 | 8 | 12 | 30 | 26 | 11 | 88 |
| CSW643xAurora | 0 | 0 | 18 | 9 | 29 | 28 | 19 | 103 |
| CSW643xRoyalRed | 1 | 0 | 6 | 54 | 77 | 21 | 30 | 189 |
| CSW643xTenderCrop | 1 | 0 | 7 | 18 | 46 | 31 | 55 | 158 |
| CSW643xPoamoho | 10 | 0 | 25 | 23 | 36 | 8 | 34 | 136 |
| CSW643xHwnWonder | 89 | 0 | 1 | 6 | 19 | 4 | 48 | 167 |
| RoyalRedxOlathe | 69 | 0 | 2 | 2 | 13 | 7 | 14 | 107 |
| RoyalRedxPinto650 | 19 | 0 | 4 | 20 | 11 | 4 | 28 | 86 |
| RoyalRedxAurora | 43 | 0 | 3 | 4 | 12 | 3 | 14 | 79 |
| RoyalRedxBushRoman | 10 | 0 | 2 | 3 | 2 | 0 | 20 | 37 |
| KW765xAurora | 4 | 0 | 0 | 8 | 27 | 3 | 4 | 46 |
| OlathexPoamoho | 83 | 0 | 7 | 18 | 25 | 34 | 39 | 206 |
| OlathexHwnWonder | 49 | 0 | 0 | 0 | 0 | 0 | 1 | 50 |
| OlathexAstro | 46 | 0 | 0 | 0 | 6 | 1 | 7 | 60 |

Appendix E (Continued)
$F_2$ Segregation for Resistance to H2

| Parents | Reaction Class[z] I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|
| OlathexAurora | 77 | 0 | 0 | 0 | 2 | 1 | 30 | 110 |
| TenderCxBushRomano | 28 | 0 | 5 | 0 | 7 | 1 | 16 | 57 |
| TenderCxTidalWave | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 18 |
| AuroraxPinto650 | 6 | 0 | 0 | 0 | 1 | 0 | 46 | 53 |
| AuroraxTenderCrop | 4 | 0 | 0 | 0 | 0 | 9 | 41 | 54 |

[z] I = I of Stavely (no visible symptoms)
II = HR of Stavely but includes necrotic spots with size 3, 4 or 5 pustules
III = R of Stavely (3, 34)
IV = MR of Stavely (4, 43)
V = MS of Stavely (345, 45, 435, etc.)
VI = S of Stavely (456, 546, 564, etc.)
VII = VS of Stavely (6, 65, 654)

Appendix F
$F_2$ Segregation for Resistance to H3

| Parents | Reaction Class[z] I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|
| Ec299xCSW643 | 38 | 0 | 12 | 52 | 13 | 1 | 13 | 129 |
| Ec299xPinto650 | 3 | 1 | 65 | 34 | 12 | 21 | 23 | 159 |
| Ec299xBush Romano | 5 | 0 | 51 | 58 | 7 | 3 | 28 | 152 |
| Ec299xTenderCrop | 21 | 0 | 45 | 27 | 3 | 5 | 10 | 111 |
| Ec299xAurora | 3 | 0 | 88 | 42 | 5 | 21 | 30 | 189 |
| Ec299xOlathe | 3 | 0 | 79 | 17 | 6 | 10 | 19 | 134 |
| Ec299xRoyalRed | 37 | 0 | 25 | 9 | 3 | 2 | 6 | 82 |
| Ec299xCNC | 3 | 0 | 99 | 81 | 11 | 15 | 2 | 211 |
| Ec299xMexico309 | 24 | 0 | 102 | 9 | 0 | 0 | 0 | 135 |
| Ec299xAxS37 | 26 | 0 | 8 | 2 | 0 | 0 | 0 | 36 |
| AxS37xCNC | 11 | 0 | 22 | 9 | 10 | 3 | 4 | 59 |
| AxS37xAstro | 9 | 0 | 12 | 14 | 3 | 3 | 7 | 48 |
| AxS37xBushRomano | 6 | 0 | 9 | 14 | 2 | 0 | 9 | 40 |
| AxS37xTenderCrop | 4 | 0 | 9 | 5 | 10 | 0 | 8 | 36 |
| Mex235xOlathe | 7 | 0 | 9 | 1 | 0 | 0 | 0 | 17 |
| Mex235xCSW643 | 20 | 0 | 5 | 8 | 4 | 0 | 5 | 42 |
| NEP-2xOlathe | 16 | 0 | 58 | 20 | 42 | 25 | 13 | 174 |
| NEP-2xPoamoho | 3 | 0 | 12 | 20 | 51 | 58 | 50 | 194 |
| NEP-2xHwnWonder | 4 | 0 | 19 | 10 | 13 | 9 | 50 | 105 |
| NEP-2xPinto650 | 0 | 0 | 6 | 4 | 9 | 0 | 7 | 26 |
| Mex309xAurora | 21 | 0 | 99 | 30 | 1 | 3 | 42 | 196 |
| Mex309xOlathe | 0 | 0 | 54 | 24 | 14 | 16 | 31 | 139 |
| Mex309xCSW643 | 18 | 0 | 92 | 29 | 7 | 8 | 39 | 193 |
| Mex309xCNC | 52 | 0 | 104 | 18 | 3 | 1 | 2 | 180 |
| CNCxAurora | 3 | 0 | 27 | 57 | 0 | 14 | 10 | 111 |
| CNCxCSW643 | 1 | 0 | 0 | 4 | 8 | 8 | 10 | 31 |
| CNCxTenderCrop | 0 | 0 | 0 | 0 | 3 | 12 | 25 | 40 |
| CNCxG.G.Wax | 8 | 0 | 0 | 0 | 1 | 5 | 18 | 32 |
| OlathexAurora | 0 | 0 | 0 | 0 | 1 | 0 | 109 | 110 |
| OlathexCSW643 | 0 | 0 | 0 | 0 | 8 | 52 | 33 | 93 |
| OlathexPoamoho | 0 | 0 | 0 | 0 | 13 | 46 | 147 | 206 |
| OlathexHwnWonder | 3 | 0 | 0 | 0 | 2 | 5 | 40 | 50 |
| OlathexRoyalRed | 2 | 0 | 0 | 0 | 3 | 9 | 93 | 107 |
| OlathexAstro | 0 | 0 | 0 | 0 | 0 | 4 | 56 | 60 |
| CSW643xPinto650 | 1 | 0 | 0 | 3 | 37 | 47 | 119 | 207 |
| CSW643xAurora | 2 | 0 | 0 | 7 | 42 | 27 | 25 | 103 |
| CSW643xRoyalRed | 0 | 0 | 0 | 12 | 71 | 55 | 51 | 189 |
| CSW643xTenderCrop | 0 | 0 | 0 | 0 | 12 | 42 | 104 | 158 |
| CSW643xPoamoho | 2 | 0 | 0 | 6 | 22 | 9 | 97 | 136 |
| CSW643xHwnWonder | 5 | 0 | 0 | 0 | 31 | 16 | 115 | 167 |
| RoyalRedxPinto650 | 0 | 0 | 0 | 3 | 7 | 4 | 72 | 86 |
| RoyalRedxAurora | 20 | 0 | 3 | 10 | 18 | 4 | 24 | 79 |
| RoyalRedxBushRomano | 0 | 0 | 0 | 0 | 4 | 1 | 32 | 37 |
| TenderCxTidalWave | 5 | 0 | 1 | 0 | 0 | 0 | 12 | 18 |
| TenderCxBushRomano | 0 | 0 | 0 | 0 | 1 | 12 | 44 | 57 |

Appendix F (Continued)
$F_2$ Segregation for Resistance to H3

| Parents | Reaction Class[z] I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|
| KW765xAurora | 0 | 0 | 0 | 1 | 10 | 14 | 21 | 46 |
| AuroraxPinto650 | 3 | 0 | 0 | 0 | 0 | 0 | 50 | 53 |
| AuroraxTenderCrop | 1 | 0 | 0 | 2 | 0 | 5 | 46 | 54 |

[z] I = I of Stavely (no visible symptoms)
II = HR of Stavely but includes necrotic spots with size 3, 4 or 5 pustules
III = R of Stavely (3, 34)
IV = MR of Stavely (4, 43)
V = MS of Stavely (345, 45, 435, etc.)
VI = S of Stavely (456, 546, 564, etc.)
VII = VS of Stavely (6, 65, 654)

Appendix G
$F_2$ Segregation for Resistance to H4

| Parents | Reaction Class[z] I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|
| Ec299xCSW643 | 7 | 0 | 48 | 7 | 10 | 1 | 4 | 77 |
| Ec299xPinto650 | 9 | 0 | 39 | 11 | 2 | 13 | 5 | 79 |
| Ec299xAurora | 0 | 0 | 56 | 9 | 0 | 16 | 11 | 92 |
| Ec299xOlathe | 21 | 0 | 37 | 4 | 3 | 3 | 6 | 74 |
| Ec299xCNC | 0 | 0 | 61 | 9 | 19 | 0 | 2 | 91 |
| Ec299xMexico309 | 1 | 0 | 89 | 0 | 1 | 0 | 0 | 91 |
| Mex309xAurora | 1 | 0 | 55 | 14 | 0 | 3 | 20 | 93 |
| Mex309xOlathe | 4 | 0 | 44 | 20 | 4 | 11 | 13 | 96 |
| Mex309xCSW643 | 4 | 0 | 53 | 19 | 6 | 0 | 10 | 92 |
| Mex309xCNC | 0 | 0 | 77 | 0 | 8 | 3 | 1 | 89 |
| CNCxCSW643 | 2 | 0 | 42 | 11 | 14 | 4 | 0 | 73 |
| NEP-2xOlathe | 0 | 0 | 38 | 6 | 21 | 19 | 7 | 91 |
| NEP-2xPoamoho | 0 | 0 | 3 | 6 | 12 | 30 | 27 | 78 |
| Mex235xOlathe | 1 | 0 | 30 | 3 | 7 | 10 | 0 | 51 |
| OlathexAurora | 0 | 0 | 0 | 10 | 24 | 27 | 3 | 64 |
| OlathexCSW643 | 1 | 0 | 1 | 24 | 49 | 18 | 0 | 93 |
| OlathexPoamoho | 0 | 0 | 0 | 0 | 17 | 42 | 31 | 90 |
| CSW643xPinto650 | 1 | 0 | 1 | 5 | 29 | 42 | 11 | 89 |
| CSW643xRoyalRed | 0 | 0 | 5 | 32 | 37 | 25 | 28 | 127 |
| CSW643xPoamoho | 8 | 0 | 0 | 0 | 6 | 7 | 29 | 50 |
| CSW643xHwnWonder | 0 | 0 | 2 | 1 | 7 | 14 | 36 | 60 |
| RoyalRedxBushRoman | 11 | 0 | 0 | 0 | 2 | 0 | 24 | 37 |

[z] I = I of Stavely (no visible symptoms)
II = HR of Stavely but includes necrotic spots with size 3, 4 or 5 pustules
III = R of Stavely (3, 34)
IV = MR of Stavely (4, 43)
V = MS of Stavely (345, 45, 435, etc.)
VI = S of Stavely (456, 546, 564, etc.)
VII = VS of Stavely (6, 65, 654)

## LITERATURE CITED

Allen, D.J. 1975. Induced resistance to bean rust and its possible epidemiological significance. Annual Report of the Bean Improvement Cooperative 18:15.

Allen, D.J. 1982. *Verticillium lecanii* on the bean rust fungus, *U. appendiculatus*. Transactions of the British Mycological Society 79:362-364.

Alten, H. von. 1983. The effect of temperature, light, leaf age on the frequency of appressoria formation and infection with *U. phaseoli* (Pers.) Wint. Phytopathology 107:327-335.

Arthur, J.C. 1962. Manual of rusts in the United States and Canada. Hafner Publishing Co., New York.

Augustin, E., D.P. Coyne, and M.L. Schuster. 1972. Inheritance of resistance in *Phaseolus vulgaris* to *Uromyces phaseoli typica* Brazilian rust race B11 and of plant habit. J. Amer. Soc. Hort. Sci. 96:526-529.

Ayala, F.J. 1982. Population and evolutionary genetics: a primer. The Benjamin/Cummings Publishing Company, Inc., California.

Baker, C.J., J.R. Stavely, and N. Mock. 1985. Biocontrol of bean rust by *Bacillus subtilis* under field conditions. Plant Disease 69:770-772.

Ballantyne, B. 1974. Resistance to rust (*U. appendiculatus*) in beans (*Phaseolus vulgaris*). Proceedings of the Linnean Society of New South Wales 98:107-119.

Ballantyne, B. 1976. Resistance to rust, caused by *U. appendiculatus* in bean. Annual Report of the Bean Improvement Cooperative 19:15-16.

_____. 1981. Bean Program Annual Report. CIAT. Cali, Colombia.

_____. 1984. Bean Program Annual Report. CIAT. Cali, Colombia.

Bernier, C.C. and R.L. Conner. 1982. Breeding for resistance to faba bean rust, p. 251-257. In: G. Hawtin and C. Webb (eds.). Faba bean improvement. Martinus Nijhoff Publishers, The Netherlands.

Bessey, E.A. 1950. Morphology and taxonomy of fungi. The Blakiston Company. Philadelphia.

Bliss, F.A. 1980. Common bean, p. 273-283. In: W.R. Fehr and H.H. Hadley (eds.). Hybridization of crop plants. Amer. Soc. Agron. and Crop Sci. Soc. Amer., Wisconsin, U.S.A.

Bookbinder, M.G. and J.R. Bloom. 1980. Interaction of *Uromyces phaseoli* and *Meloidogyne incognita* on bean (*Phaseolus vulgaris*). Journal of Nematology 12:177-182.

Bravo, G.H. and G. Galvez. 1976. Inheritance of disease resistance, *Phaseolus vulgaris*, in the tropics. I. Rust, common bean mosaic virus and common bacterial blight. Annual Report of the Bean Improvement Cooperative 19:47-48.

Buchenauer, H. 1982. Chemical and biological control of cereal rusts, p. 246-279. In: K.J. Scott and A.K. Chakravorty (eds.). The rust fungi. Academic Press Inc., London.

Carvalho, L.P. de, C. Vieira, and G.M. Chaves. 1978. Inheritance of resistance to five physiologic races of bean rust. Annual Report of the Bean Improvement Cooperative 21:49-50.

Christ, B.J. and J.V. Groth. 1982a. Inheritance of resistance in three cultivars of beans to the bean rust pathogen and the interaction of virulence and resistance genes. Phytopathology 72:771-773.

Christ, B.J. and J.V. Groth. 1982b. Inheritance of virulence to three bean cultivars in three isolates of the bean rust pathogen. Phytopathology 72:767-770.

Code, J.L., J.A.G. Irwin, and A. Barnes. 1985. Comparative etiological and epidemiological studies on rust diseases of *Phaseolus vulgaris* and *Macroptilium atropurpureum*. Australian Journal of Botany 33:147-157.

Cohen, Y. and J. Rotem. 1970. The relationship of sporulation to photosynthesis in some obligatory and facultative parasites. Phytopathology 60:1600-1604.

Cummins, G.B. and Y. Hiratsuka. 1983. Illustrated genera of rust fungi. Amer. Phyto. Soc., St. Paul, Minnesota.

Davison, A.D. and E.K. Vaughn. 1963. A simplified method for identification of races of *U. phaseoli* var. *phaseoli*. Phytopathology 53:456-459.

Day, P.R. 1973. The taxonomy of physiologic races, pp. 164-169. In: C.V. Subramanian (ed.). Taxonomy of fungi. Part One. University of Madras, India.

De Quattro, J. 1992. Rust-no-more beans. Agricultural Research Feb.:12-15.

Ebrahim-Nesbat, F., H.H. Hoppe, and R. Heitefuss. 1982. Ultrastructural studies on the development of *Uromyces phaseoli* in bean leaves protected by elicitors of phytoalexin accumulation. Phytopathology 103:261-271.

El Naghy, M.A. and R. Heitefuss. 1976. Permeability changes and production of antifungal compounds in *Phaseolus vulgaris* infected with *Uromyces phaseoli*. II. Role of phytoalexins. Physiological Plant Pathology 8:269-277.

Epstein, L., L. Laccetti, R.C. Staples, H.C. Hoch, and W.A. Hoose. 1985. Extracellular proteins associated with induction of differentiation in bean rust uredospore germlings. Phytopathology 75:1073-1076.

Eriksson, J. 1930. Fungous diseases of plants. 2nd ed. Bailliere, Tindall and Cox, London.

Evrard, P. and P. Lepeivre. 1983. Effect of triphenylbismuth dichloride on haricot bean rust and broad bean rust. Parasitica 39:117-127.

Farina, G., N. Barbieri, M. Bassi, and E. Betto. 1981. Heat sensitivity of mycelium and haustoria in bean leaves infected with bean rust. Mycopathologia 73:121-125.

Franje, N.S. and F.C. Quebral. 1980. The effect of rust on the photosynthetic activity of soybean (*Glycine max* (L.) Merr.). CMU Journal of Agriculture, Food and Nutrition 2:61-77.

Frazier, W.A. and J.W. Hendrix. 1949. Hawaiian Wonder. University of Hawaii Agricultural Experiment Station. Oct.

Freytag, G.F., J.D. Kelly, M.W. Adams, J. Lopez Rosa, J. Beaver and R. Echavez Badel. 1985. Registration of two navy bean germplasm lines L226-10 and L227-1. Crop Science 25:714.

Gjærum, H.B. 1985. East African rusts (Uredinales), mainly from Uganda. Mycotaxon 24:237-273.

Gold, R.E. and K. Mendgen. 1983a. Activation of teliospore germination in *U. appendiculatus* var. *appendiculatus*. II. Light and host volatiles. Phytopathologische Zeitschrift 108:281-293.

Gold, R.E. and K. Mendgen. 1983b. Activation of teliospore germination in *Uromyces appendiculatus* var. *appendiculatus*. I. Aging and temperature. Phytopathologische Zeitschrift 108:267-280.

Goode, M.J. 1961. A new race of bean rust in Arkansas. Plant Disease Reporter 45:690-691.

Grafton, K.F., G.C. Weiser, L.J. Littlefield, and J.R. Stavely. 1985. Inheritance of resistance to two races of leaf rust in dry edible bean. Crop Science 25:537-539.

Groth, J.V. and B.D. Mogen. 1978. Completing the life cycle of *Uromyces phaseoli* var. *typica* on bean plants. Phytopathology 68:1674-1677.

Groth, J.V. and W.J. Shrum. 1977. Virulence in Minnesota and Winsconsin bean rust collections. Plant Disease Reporter 61:756-760.

Harter, L.L. and W.J. Zaumeyer. 1941. Differentiation of physiologic races of *Uromyces phaseoli typica* on bean. Journal of Agricultural Research 62:717-731.

Hartmann, R.W. 1984. `Poamoho' pole bean. University of Hawaii. HITAHR. Research Extension Series 051. Nov.

Hartwick, N.V., A.D. Greenwood, and R.K.S. Wood. 1975. Observations on the structure of uredospores of *Uromyces appendiculatus*. Trans. Br. Mycol. Soc. 64:289-293.

Heath, M.C. 1972. Ultrastructure of host and nonhost reactions to cowpea rust. Phytopathology 62:27-38.

Heath, M.C. 1974. Light and electron microscope studies of the interactions of host and nonhost plants with cowpea - *Uromyces phaseoli* var. *vignae*. Physiological Plant Pathology 4:403-414.

Heath, M.C. 1976. Hypersensitivity, the cause or the consequence of rust resistance? Phytopathology 66:935-936.

Heath, M.C. 1981. Resistance of plants to rust infection. Phytopathology 71:971-974.

Heath, M.C. 1982. Host defense mechanisms against infection by rust fungi, p. 223-245. In: K.J Scott and A.K. Chakravorty (eds.). The rust fungi. Academic Press Inc., London.

Hill, J.P., H.F. Schwartz, M. Salgado, and R.E. Farrera. 1990. Effects of sub-lethal applications of propiaconazole on bean rust. Annual Report of the Bean Improvement Cooperative 33:187-188.

Hiratsuka, Y. and S. Sato. 1982. Morphology and taxonomy of rust fungi, p. 1-36. In: K.J. Scott and A.K. Chakravorty (eds.). The rust fungi. Academic Press Inc., London.

Hoch, H.C. and R.C. Staples. 1984. Evidence that cAMP initiates nuclear division and infection structure-formation in the bean rust fungus, *U. phaseoli*. Experimental Mycology 8:37-46.

Hoppe, H.H., B. Humme, and R. Heitefuss. 1980. Elicitor induced accumulation of phytoalexins in healthy and rust infected leaves of *P. vulgaris*. Phytopathologische Zeitschrift 97:85-88.

Hubbeling, N. 1957. New aspects of breeding for disease resistance in beans (*Phaseolus vulgaris* L.). Euphytica 6:111-141.

Imhoff, M.W., C.E. Main, and K.J. Leonard. 1981. Effect of temperature, dew period and age of leaves, spores and source pustules on germination of bean rust urediospores. Phytopathology 71:577-583.

Immer, F.R. 1930. Formulae and tables for calculating linkage intensities. Genetics 15:81-98.

_____. 1977. International bean rust nursery results 1975-1976. CIAT, Cali, Colombia.

_____. 1979. International bean rust nursery results 1977-1978. CIAT, Cali, Colombia.

Johnson, R. and D.J. Allen. 1975. Induced resistance to rust diseases and its possible role in the resistance of multiline varieties. Annals of Applied Biology 80:359-363.

Kaminskyj, S.G.W. and M.C. Heath. 1982. An evaluation of the nitrous acid-3-methyl-2-benzothiazolinone hydrazone hydrochloride-ferric chloride assay for chitin in rust fungi and rust-infected tissue. Canadian Journal of Botany 60:2575-2580.

Kaminskyj, S.G.W. and A.W. Day. 1984. Chemical induction of infection structures in rust fungi. 1. Sugars and complex media. Experimental Mycology 8:63-72.

Kolmer, J.A. and J.V. Groth. 1984. Inheritance of a minute uredinium infection type of bean rust in bean breeding line 814. Phytopathology 74:205-207.

Martin, J.H. and W.H. Leonard. 1970. Principles of field crop production. 2nd ed. McMillan Co., New York.

McIntosh, R.A. and I.A. Watson. 1982. Genetics of host-pathogen interactions in rusts, p.121-149. In: K.J. Scott and A.K. Chakravorty (eds.). The rust fungi. Academic Press Inc., London.

Meiners, J.P. 1979. Sources of resistance to U.S. bean rust - update. Annual Report of the Bean Improvement Cooperative 22:62-63.

Meiners, J.P. 1980. Results - uniform snap bean rust nursery - 1979. Annual Report of the Bean Improvement Cooperative 23:29-30.

Meiners, J.P. 1981. Genetics of disease resistance in edible legumes. Ann. Rev. Phytopathol 21:189-209.

Meiners, J.P., C.W. Rogers, and F.D. Schales. 1975. Field tests in Maryland for rust resistance in dry beans. Annual Report of the Bean Improvement Cooperative 18:46-47.

Mendgen, K. 1973. Electronmicroscopy of the bean rust infection structures. Phytopathologische Zeitschrift 78:109-120.

Menten, J.O.M. and A.B. Filho. 1981. Monocycle components of beans (*P. vulgaris* L.) resistance to *U. appendiculatus* and their relationship with the epidemiological parameters Xo and r. Annual Report of the Bean Improvement Cooperative 24:6.

Mmbaga, M.T. and J.R. Stavely. 1988. Pathogenic variability in *Uromyces appendiculatus* from Tanzania and rust resistance in Tanzanian bean cultivars. Plant Disease 72:259-262.

Mmbaga. M.T. and J.R. Steadman. 1991a. Adult plant resistance in common beans. Annual Report of the Bean Improvement Cooperative 34:41-42.

Mmbaga, M.T. and J.R. Steadman. 1991b. Rust urediniospore production and release in relation to leaf pubescence in common beans. Annual Report of the Bean Improvement Cooperative 34:43-44.

Mmbaga, M.T., E. Arnaud-Santana, J.R. Steadman and D. Coyne. 1991. Reaction of Pompadour bean lines to *Uromyces appendiculatus* and *Xanthomonas campestris* pv. *phaseoli*. Annual Report of the Bean Improvement Cooperative 34:45-46.

Montalbini, P. and E.F. Elstner. 1977. Ethylene evolution by rust-infected, detached bean (*P. vulgaris*) leaves susceptible and hypersensitive to *U. phaseoli*. Planta 135:301-306.

Muchovej, J.J. and D.D. Dhingra. 1980. Acetone, benzene and ethanol for treating *Phaseolus* bean seeds in the dry state with systemic fungicides. Seed Science and Technology 8:351-356.

Muller, L.Y., F.H.J. Rijkenberg, and S.J. Truter. 1974. A preliminary ultrastructural study on the *Uromyces appendiculatus* teliospore stage. Phytophylactica 6:123-128.

Mullins, C.A. and S.C. Bost. 1991. Fungicides for rust control in Tennessee. Annual Report of the Bean Improvement Cooperative 34:49.

Omunyin, M.E., H.A.I. Stoetzer, and J.N. Kimamira. 1984. Field infection of beans with bean rust. Annual Report of the Bean Improvement Cooperative 27:94.

Parris, G.K. and M. Matsuura. 1941. A second strain of bean rust in Hawaii. Plant Disease Reporter 25:311.

_____. 1968-82. Plant Disease Clinic Report. Cooperative Extension Service. University of Hawaii.

Pring, R.J. 1980. A fine-structural study of the infection of leaves of *Phaseolus vulgaris* by uredospores of *Uromyces phaseoli*. Physiological Plant Pathology 17:269-276.

Pring, R.J. and D.V. Richmond. 1976. An ultrastructural study of the effect of oxycarboxin on *Uromyces phaseoli* infecting leaves of *Phaseolus vulgaris*. Physiological Plant Pathology 8:155-162.

Ramakrishnan, L. and R.C. Staples. 1970. Changes in ribonucleic acids during uredospore differentiation. Phytopathology 60:1087-1091.

Rolim, P.R.R., F.B. Neto, and A.J. Roston. 1981. Chemical control of bean (*Phaseolus vulgaris*) diseases. I. Rust (*Uromyces phaseoli*). Biologico 47:201-205.

Rudolph, K. and N. Baykal. 1978. Diseases of beans (*Phaseolus vulgaris*) in south and west Turkey. Annual Report of the Bean Improvement Cooperative 21:45-47.

Rusuku, G., P. Lepoivre, M. Meulemans, and J. Semal. 1984. Effects of triphenylphosphite on bean rust development. Plant Disease 68:154-155.

Savile, D.B.O. 1979. The evolution of anamorphs in the Uredinales, p.547-554. In: B. Kendrick (ed.). The whole fungus. Vol. II. National Museum of Natural Sciences, National Museums of Canada and the Kananaskis Foundation, Canada.

Schein, R.D. 1961. Temperature conversion of rust response of beans. Phytopathology 51:486-488.

Schein, R.D. 1962. Storage viability of bean rust uredospores. Phytopathology 52(7):653-657.

Schein, R.D. 1965. Age-correlated changes in susceptibility of bean leaves to *U. phaseoli* and tobacco mosaic virus. Phytopathology 55:454-457.

Schwartz, H.F. and S.R. Temple. 1978. Bean rust resistance strategy at CIAT. Annual Report of the Bean Improvement Cooperative 21:48-49.

Sempio, C., G. Della Torre, F. Ferranti, B. Barberini, and R. Draoli. 1975. Defence mechanism in beans resistant to rust. Phytopathologische Zeitschrift 83:244-266.

Shaik, M. 1985a. Races of the bean rust fungus, *U. appendiculatus* var. *appendiculatus*, from Jamaica. Annual Report of the Bean Improvement Cooperative 28:20-21.

Shaik, M. 1985b. Race-nonspecific resistance in bean cultivars to races of *U. appendiculatus* var. *appendiculatus* and its correlation with leaf epidermal characteristics. Phytopathology 75:478-481.

Shaik, M. and J.R. Steadman. 1988. Nonspecific resistance to bean rust and its association with leaf pubescence. Annual Report of the Bean Improvement Cooperative 31:62-63.

Small, B.C., M.D. Montgillion, and T.G. Pridham. 1961. Phleomycin, an antibiotic markedly effective for control of bean rust. Plant Disease Reporter 45:244-247.

Snel, M. and L.V. Edgington. 1969. Decomposition and distribution of labeled oxathiin fungicides, systemic in bean. Phytopathology 59:1050. (Abstr.)

Snel, M. and L.V. Edgington. 1970. Uptake, translocation, and decomposition of systemic oxathiin fungicide in bean. Phytopathology 60:1708-1716.

Staples, R.C. 1974. Synthesis of DNA during differentiation of bean rust uredospores. Physiological Plant Pathology 4:415-424.

Staples, R.C. and H.C. Hoch. 1982. A possible role for microtubules and microfilaments in the induction of nuclear division in bean rust uredospore germlings. Experimental Mycology 6:293-302.

Stavely, J.R. 1983. A rapid technique for inoculation of *Phaseolus vulgaris* with pathotypes of *Uromyces phaseoli*. Phytopathology 73:676-679.

Stavely, J.R. 1984a. The 1983 bean rust nurseries. Annual Report of the Bean Improvement Cooperative 27:100-102.

Stavely, J.R. 1984b. Pathogenic specialization in *U. phaseoli* in the U.S. and rust resistance in beans. Plant Disease 68:95-99.

Stavely, J.R. 1984c. Pathogenic specialization in *U. phaseoli* and rust resistance in bean germplasm. Annual Report of the Bean Improvement Cooperative 27:14-15.

Stavely, J.R. 1988. Rust resistance in beans: the plant introduction collection as a resource and resistance development. Annual Report of the Bean Improvement Cooperative 31:64-65.

Stavely, J.R. and J. Steinke. 1985. BARC-Rust Resistant -2, -3, -4, and -5 snap bean germplasm. HortScience 20:779-780.

Stavely, J.R., G.F. Freytag, J.R. Steadman, and H.F. Schwartz. 1983. The 1983 bean rust workshop. Annual Report of the Bean Improvement Cooperative 26:iv-vi.

Stavely, J.R., J. Steinke, R.T. McMillan Jr., K.F. Grafton, J.R. Steadman, J.D. Kelly, D.P. Coyne, D.T. Lindgren and M.J. Silbernagel. 1992. Rust resistant bean germplasm releases. Annual Report of the Bean Improvement Cooperative 35:228-229.

Vargas, E. 1980. Rust, p. 17-36. In: H.F. Schwartz and G.E. Galvez (eds.). Bean production problems. CIAT, Colombia.

Vaughn, E.K. 1974. Field testing for bean rust resistance. Annual Report of the Bean Improvement Cooperative 17:89-91.

Wolf, G. 1982. Physiology and biochemistry of spore germination, p. 151-178. In: K.J. Scott and A.K. Chakravorty (eds.). The rust fungi. Academic Press inc., London.

Wood, D.R. and J.G. Keenan. 1982. Registration of Olathe pinto beans. Crop Science 22:1259-1260.

Wynn, W.K. 1976. Appressorium formation over stomates by the bean rust fungus: response to a surface contact stimulus. Phytopathology 66:136-146.

Yarwood, C.E. 1956. Cross protection with two rust fungi. Phytopathology 46:540-544.

Yarwood, C.E. 1965. Predisposition to mildew by rust infection, heat, abrasion and pressure. Phytopathology 55:1372.

Yarwood, C.E. 1978. Translocated heat therapy of bean rust. Phytopathology 68:1170-1171.

Yen, D.E. and R.M. Brien. 1960. French bean rust (*U. appendiculatus*) studies on resistance and determination of rust races present in New Zealand. New Zealand Journal of Agricultural Research 3:358-363.

Zaiter, H.Z., D.P. Coyne, and J.R. Steadman. 1990. Coinoculation effects of the pathogens causing common bacterial blight, rust, and bean common mosaic in *Phaseolus vulgaris*. J. Amer. Soc. Hort. Sci 115:319-323.

Zaumeyer, W.J. and H.R. Thomas. 1957. A monographic study of bean diseases and methods for their control. USDA Technical Bulletin No. 868.